Author:
Henderson, Anna Catrin

Title:
Studies on interactions among Xenopus taxa using comparative osteology and other methods : an evolutionary perspective

STUDIES ON INTERACTIONS AMONG *XENOPUS* TAXA USING COMPARATIVE OSTEOLOGY AND OTHER METHODS: AN EVOLUTIONARY PERSPECTIVE

Anna Henderson

A dissertation submitted to the University of Bristol in accordance with the requirements of the degree of Doctor of Philosophy in the Faculty of Science.

August, 2002

68,166 words

CONTAINS DISKETTE

UNABLE TO COPY

CONTACT UNIVERSITY

IF YOU WISH TO SEE

THIS MATERIAL

ABSTRACT

The past thirty years have seen a tripling in the number of *Xenopus* species recognized. The complex pattern of species distribution demonstrated across the sub-Saharan range reflects ecological constraints imposed by climate conditions throughout the Pleistocene, and highlights a number of areas of specific biogeographic interest.

Although previous application of comparative osteology as a method for phylogenetic investigation at the species level in Anura has been limited, the approach, adopted in this study, has proven to be highly informative in its application to specific taxonomic studies.

An overview of osteological characters in the type species of the genus *Xenopus* is presented for the first time and forms the basis for subsequent osteology-based species descriptions. Results implicate two tetraploids, *X. l. victorianus* and *X. fraseri* in the hybrid origin of an octoploid species pair, *X. wittei* and *X. vestitus* in the Central African highlands. Existing ambiguity surrounding the taxonomic distinction between two cryptic taxa, also living in these highlands, *X. l. victorianus* and *X. l. bunyoniensis* is considered. Osteologically, the two are distinct.

Corroboration for the sub-division of *X. muelleri* into eastern and western forms is provided by studies on comparative osteology and mating-call, amongst others. The taxonomic significance of these findings is overshadowed however, by evidence of widespread hybridization and introgression with distantly related *X. l. laevis* in the southeastern part of the *X. muelleri* range.

Although only two species are currently known from Ethiopia, evidence of morphology indicates that museum collections of *X. clivii* comprise at least two distinct forms, one appearing to be intermediate between the two *Xenopus* sub-genera. Furthermore, osteology reveals that *X. largeni* also straddles this taxonomic boundary, casting doubt on the robustness of this phylogenetic division. Results are consistent with recent findings from molecular-based phylogenies, qualifying the use of comparative osteology as an informative method for investigation of evolutionary interactions.

This thesis is dedicated to the memory of Dr. Raymond F. Laurent.

ACKNOWLEDGEMENTS

Principally, I would like to extend my gratitude to Richard Tinsley for his advice, time and patience. Thanks are due also to all those who have helped with lab work, Drs. Joe Jackson, Steve Beard, Mick Bailey, Stuart Parsons, Dave Goodson (University of Loughborough), Marc Holderied, and Bob Porter, Nigel Ashcroft at Green Umbrella, Tim Colburn, John Glew (Long Ashton Dental Surgery), those at the University of Bristol School of Veterinary Science, Anna Hobbs and Steve Martin. For assistance with fieldwork, thanks to Dr. Louis DuPreez, Richard Boycott (Malolotja Nature Reserve, Senior Warden), Dr. Andrew Deacon (Kruger Nature Reserve, Warden), Mr. Fouche and to farmers who gave permission to collect on their land. Special thanks to Dr. Marissa Fabrezi at Salta Museum of Natural History for her encouragement, to Barry Clarke at the British Museum of Natural History, Linda Ford at the American Museum of Natural History and Musée Royal de l'Afrique Centrale, Tervuren, Belgium for loan of specimens.

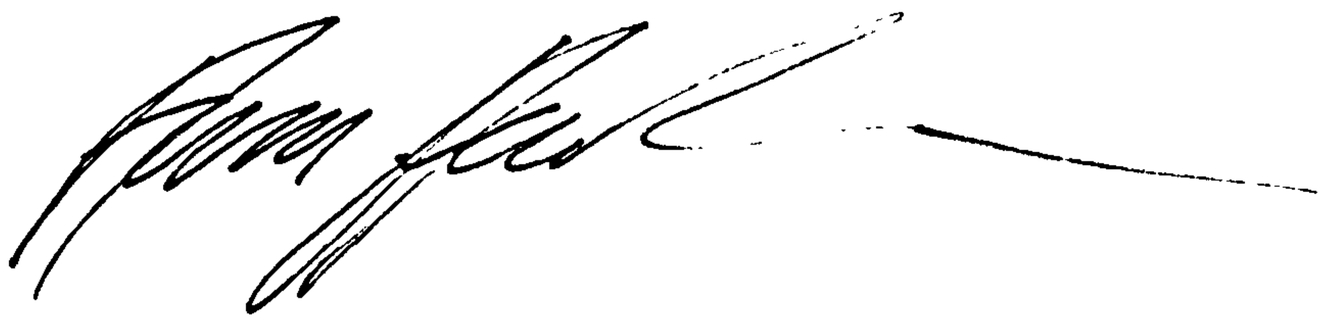
I am indebted to those in the lab, Emily, Miguel and Sinead for their support and friendship, and to those at home Paul, Bridie and Pete for tolerating grumbles, and of course to Rob who, since the very first balloon took to the air, has provided immeasurable support.

Thanks also to Elin, Nia, Ma, Pa and Nain, for their continued support and encouragement.

Finally I thank the University for scholarship funding.

AUTHOR'S DECLARATION

I declare that the work in this dissertation was carried out in accordance with the Regulations of the University of Bristol. The work is original except where indicated by special reference in the text and no part of the dissertation has been submitted for any other degree. Any views expressed in the dissertation are those of the author and in no way represent those of the University of Bristol. The dissertation has not been presented to any other University for examination either in the United Kingdom or overseas.



Anna Catrin Henderson
August, 2002

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 8

LIST OF ILLUSTRATIONS

CHAPTER 2

CHAPTER 3

CHAPTER 4

LIST OF TABLES

CHAPTER 7

CHAPTER 1 : THESIS INTRODUCTION

Xenopus is an aquatic amphibian (family Pipidae: Anura) of Gondwanan origin (Henrici and Baez, 2001). Extant species of the genus occur in Africa primarily south of the Sahara, but the ranges of two taxa extend further north, with isolated populations occurring in Jebel Marra, Sudan and in the Ennedi, Chad (Tinsley, Loumont and Kobel, 1996). One taxon, *Xenopus laevis laevis* is widely used in laboratories, being investigated at molecular, cellular and developmental levels (Cannatella and De Sa, 1993; Henrici and Baez, 2001). Used first in the 1930's as an assay for human pregnancy, the group lends itself to such research uses owing to its fully aquatic lifestyle, resistance to infection and disease, short life cycle and large oocytes (Cannatella and De Sa, 1993; Gurdon, 1996). Although most lab-based research has focused on a single taxon, *X. l. laevis*, studies on other *Xenopus* species have led to the discovery that their chromosome complements form a polyploid series (Kobel, Loumont and Tinsley, 1996). Polyploidy is known to have arisen through inter-species hybridization, and involves the duplication of chromosome numbers in oocytes prior to fertilization. That all except for a single species are polyploid demonstrates the importance of allopolyploidy in the evolution of the genus (Kobel, 1996). Allopolyploidy is in fact rare in animals, yet in vertebrates there is a disproportionate concentration of polyploid species in anuran amphibians (Ptacek, Gerhardt and Sage, 1994). Indeed, the genus *Xenopus* represents the species group with the greatest variation in ploidy levels known amongst vertebrates (Schmidt and Steinlein, 1991).

Despite a high degree of genetic diversity and range of ploidy levels, the genus is characterized by a highly conservative morphology making morphological distinction between many species impossible (Tinsley and Kobel, 1996). Methods employing mate-call, karyotype, DNA content, electrophoresis of proteins and experimental hybridization have each been employed to distinguish between taxa (Kobel *et al.*, 1996; Graf, 1996). These same methods have led to an increase in the number of species recognized since the early 1970's. Numbers have almost tripled in this time as a result also of more intensive collecting in remote and previously uncollected areas and the careful re-examination of existing collections (Tinsley, 1995, 1996; Henrici and Baez, 2001).

The genus is divided into two sub-genera (Kobel *et al.*, 1996), *Silurana* and *Xenopus*, which are separated on the basis of differences in basic chromosome number (De Sa and Hillis, 1990; Kobel *et al.*, 1998), this number in *Silurana* being 10, and in *Xenopus*, 9. *Silurana* comprises 2 species, diploid *(S.) tropicalis* (2n=20) and tetraploid *(S.) epitropicalis* (2n=40). *Xenopus* is represented by 15 species, varying in ploidy between tetraploid (2n=36), octoploid (2n=72) and dodecaploid (2n=108) (Tinsley *et al.*, 1996). Although there is broad consensus on taxonomic groupings within the genus with species falling into three main groups (Table 1-1), a few species defy categorization and have consistently been determined as being isolated with respect to other *Xenopus* representatives (Graf, 1996; Tinsley and Kobel, 1996; Kobel *et al.*, 1998).

Sub-genus/ group	Species	Ploidy	2n Chromosome number
Silurana	*tropicalis, epitropicalis*	Diploid Tetraploid	20 40
Xenopus muelleri	*muelleri east and west, borealis*	Tetraploid	36
Xenopus fraseri	*fraseri, pygmaeus, wittei, amieti, andrei boumbaensis ruwenzoriensis,*	Tetraploid Octoploid Dodecaploid	36 72 108
Xenopus laevis	*laevis (sub-species laevis, poweri, petersi, victorianus, sudanensis bunyoniensis), gilli*	Tetraploid	36
Taxonomically isolated *Xenopus* species	*largeni, clivii vestitus longipes*	Tetraploid Octoploid Dodecaploid	36 72 108

Table 1-1- Chromosome number and ploidy level in *Xenopus* species.

SPECIES DISTRIBUTION

Before the early 1970's, only 6 species were known. Their distribution appeared to reflect broad ecological divisions. Four were believed to occupy largely parapatric ranges, *X. muelleri* and *X. laevis* occurring in savanna, *X. fraseri* and *X. tropicalis* in lowland tropical forests. The remaining two species, *X. gilli* and *X. clivii* had restricted, possibly relict distributions in the Cape and Ethiopian highlands respectively (Tinsley *et al.*, 1996). With an increase in the number of recognized species, the actual distribution is now known to be considerably more complex (Tinsley *et al.*, 1996). Many of the recently described forms are from narrowly defined areas characterized by high diversity and endemism (Tinsley, 1973, 1975; Laurent, 1972; Tymowska and Fischberg, 1973; Tinsley *et al.*, 1979; Kobel, Du Pasquier, Fischberg and Gloor, 1980; Loumont, 1983; Loumont and Kobel, 1991). The distribution of others remains poorly defined (Loumont, 1983, 1986; Tinsley, 1995). The main peaks for species diversity and endemism are focused in the highlands of the Albertine Rift, forests of Eastern Democratic Republic of the Congo (DRC) and in lowland coastal forests and mountains of Cameroon and Gabon (Tinsley *et al.*, 1996).

Members of *Silurana, X. (S.) tropicalis* and *X. (S.) epitropicalis* are both lowland forest species, their respective ranges separated at the approximate level of Mount Cameroon (Tinsley *et al.*, 1996). *Xenopus (S.) tropicalis* is allopatric from Senegal to northern Cameroon, though the precise eastern limit of its range is undetermined (Loumont, 1984; Kobel *et al.*, 1996). The distribution of *X. epitropicalis* is less well defined, appearing to extend from southern Cameroon and Gabon into central and northern Angola. Populations have also been located in Eastern DRC, but not in southern or northern parts of the country (Loumont, 1984; Kobel *et al.*, 1996).

Xenopus fraseri is documented as occurring in the lowland forests of Nigeria, central southern Cameroon, northern Gabon and Bioko (Fernando Póo) (Boulenger, 1905; Tinsley *et al.*, 1996). The distribution of this taxon has become complicated however by the recent discovery of a number of *X. fraseri*-like cryptic taxa which are distributed throughout a range of localities that extend from lowland forests of Eastern DRC (*X. pygmaeus*) and the Semliki valley (*X. ruwenzoriensis*) to lowlands in eastern Cameroon (*X. boumbaensis*) and highlands (*X. amieti*) and coastal forests (*X. andrei*) of western Cameroon. These cryptic taxa range in ploidy from tetraploid to dodecaploid (see Table 1-1). Their relative distributions remain largely unknown.

Xenopus laevis comprises 5 sub-species (see Table 1-1; Loumont, 1984; Kobel *et al.*, 1996). The geographic ranges of these sub-species occur as a succession of broad latitudinal belts extending from the Cape in the south as far north as central Nigeria and the Jebel Marra, Sudan (Loumont, 1984; Tinsley *et al.*, 1996). One additional sub-species (*X. l. bunyoniensis*) appears to have been replaced across its former range in the central African highlands, and may have gone extinct over the course of the last century (Tinsley, 1981b). The distribution of *X. gilli,* a closely related species listed as endangered by the IUCN (International Union for Conservation of Nature) is restricted to the Fynbos habitat in the Cape of South Africa, and readily hybridizes with another Cape taxon, *X. l. laevis.*

The distribution of *X. muelleri* was originally believed to be the widest of all *Xenopus* taxa, extending in a broad arc of high temperature, low altitude grasslands from southeastern Africa and Swaziland through Botswana, Zimbabwe, Zambia, Malawi, Tanzania, northern DRC, southern Sudan, northern and southern Chad, Cameroon, Nigeria, Benin, Ghana and the Upper Volta. Evidence from recent biochemical, molecular and parasitological studies however, has suggested that distinct eastern and western forms exist, although where these ranges meet remains to be determined (Tinsley, *et al.*, 1996; Kobel *et al.*, 1996; Kobel *et al.*, 1998).

Four additional taxa fall outside these taxonomic groupings. Two are located in the Ethiopian highlands, *X. clivii* and *X. largeni.* The former taxon occurs at altitudes over 1500m in Ethiopia and Eritrea, whilst *X. largeni* is known only from the Bale Mountains in southern Ethiopia, occurring at altitudes over 2600m. The distribution of *X. vestitus* is restricted to highlands bordering Uganda, Rwanda, Burundi and eastern DRC, and that of *X. longipes*, a form that straddles the taxonomic boundary between *Silurana* and *Xenopus*, to Lake Oku in Cameroon.

In contrast to the attention attracted by *X. laevis* as a lab-model, few investigations have been conducted into the ecology of the group. Distribution studies demonstrate a distinctive biogeographic pattern that emphasizes a profound ecological division that separates forest from non-forest taxa. Fieldwork investigations have shown the requirements of forest taxa for a forest biotype to be stricter than the corresponding habitat preference expressed by non-forest taxa, the latter occasionally being

found within gallery forest (Tinsley *et al.*, 1996). The distinct distribution pattern shown by species assemblages within each of these groups is not well understood however. Since these species do not generally appear to demonstrate a preference for specific biotypes, with the exception of the forest–non-forest division and *X. gilli* in the Cape Fynbos, no obvious ecological interpretation presents itself (Tinsley, 1981b, 1996). Whilst this emphasizes the need for an increase in our understanding of their ecology and the factors responsible for their current distribution (Tinsley *et al.*, 1996). Advances in the study of African environmental history explain much of the observed pattern.

ENVIRONMENTAL HISTORY

Being Gondwanan in origin, *Xenopus* has witnessed considerable southward shift of the equator, significant restructuring of the African landscape and profound fluctuations in climate (Hamilton, 1982; Lovett, 1993). Concurrent with splitting of the super-continent approximately 100Myr BP, a previously arid African climate became increasingly moist, owing to an increase in atmospheric moisture originating from the Tethys Sea to the north and from newly formed Indian and Atlantic oceans (Lovett, 1993). Moist forest soon developed over present-day northern Africa, which at that stage lay 15-18° south of its present position, developing by the Paleocene, 60Myr BP, into lowland equatorial forest (Axelrod and Raven, 1978). Northward drift of the continent closed the Tethys Sea, reducing northern oceanic currents, and ultimately leading to fragmentation of pan-African tropical forest (Lovett, 1993). Africa continued to drift northwards for 30Myr, eventually colliding with Eurasia in the mid-Miocene, 17Myr BP, further reducing northern oceanic currents and causing a reduction in atmospheric moisture. Although most of Africa was under dry and savanna woodland by now (Chesters, 1957; Axelrod and Raven, 1978), moist forest still survived in central Africa (Lovett, 1993), the result of an increase in orographic precipitation, attributable to the developing rift system. Connection of Guineo-Congolian forests with those of East-Africa is believed to have persisted until 10Myr BP, when major uplift of the central highlands and sinking of the Congo basin altered weather patterns and drainage courses, resulting in the development of an 'arid corridor' that extended from northeast to southwestern Africa, coming east also into northern Mozambique (reviewed by Werger, 1978). This corridor presented a formidable barrier to east-west migration of forest taxa, now thought to be restricted to routes north of the Kenyan highlands or via the Lake Tanganyika rift to Lake Rukwa. Climates are believed to have become warm and humid again between 9 and 6.4Myr BP, with evidence of forest connection between western Guineo-Congolian and northwestern Ethiopian forest (Yemane, Bonnefille and Faure, 1985). By 5Myr BP, forests were again fragmented owing to ice-sheet expansion over Antarctica (Shackleton and Kennet, 1975), expanding and coalescing again during a warm wet period between 4.2 and 2.43Myr BP,

allowing migration of forest taxa between eastern and western forest habitats via the aforementioned routes (Lovett, 1993).

The whole of the African continent has since been subject to a series of dramatic climatic fluctuations driven by the expansion and contraction of ice sheets over northern parts of the northern hemisphere (Street, 1981), a cycle that has continued throughout the Pleistocene (2.5 Myr to present). This period has seen up to 21 glacial or near-glacial periods (Lovett, 1993; Wilson, 1992) accounting in total for approximately 90% of the Pleistocene period (Hamilton, 1982; Lawes, 1990; Lovett, 1993; Tinsley, 1996), the most recent dated at 18,000yr BP (Lawes, 1990; Tinsley, 1996; Jolly *et al.*, 1997, Marchant, Taylor and Hamilton, 1997). The primary effect of glacial expansion on the African sub-continent was a reduction in the availability of fresh water by as much as 30% as compared with present conditions (Bonnefille, Roeland and Guiot, 1990). Much was held in polar ice caps, causing a reduction in rates of oceanic and continental evapotranspiration. Mean temperature was also reduced by between 3°C and 6°C (Hamilton, 1982; Bonnefille and Riollet, 1988; Hamilton, 1988; Bonnefille *et al.*, 1990).

Inter-glacials, in contrast, were warmer and wetter. The last climatic optimum saw rainfall increase over the sub-continent by 125-135% compared with glacial episodes (Gasse, Tehet, Durand, Gilbert and Fontes, 1990) and has been dated at 9000-7000yr BP (Hamilton, 1972, 1976, 1982; Lovett, 1993; Tinsley, 1996). Conditions have since become slightly drier and cooler (Hamilton, 1982).

EFFECTS OF CLIMATE CHANGE ON VEGETATION

The scale and degree of impact that climate changes had on forest cover across the continent were profound. These effects would have been more pronounced in spatially uniform, un-buffered landscapes, such as those of the lowland tropics. The effects of reduction in both temperature and precipitation, especially the latter (Morrison, 1968; Hamilton, 1982; Seymour, Clerke, Channing and Crowe, 2001), were manifest in the contraction and fragmentation of lowland forests across much of the continent (Brown, 2001). During periods of glacial expansion in the northern hemisphere, the extent of lowland tropical forest was greatly reduced, giving way to savanna across much of its original range (Werger, 1978). Whilst many lowland forest species would have faced extinction (Hamilton, 1976), small, forested pockets are believed to have survived in riparian habitats along main river courses, at the base of escarpments and in coastal areas, protected from severe drought by orographic precipitation and coastal mists (Colyn, Gautierhion and Verheyen, 1991; Fjeldsa, 1994; Lovett, 1993; Tuomisto and Ruokolainene, 1997). In contrast, the range of montane forest was increased during periods of glacial expansion in the northern Hemisphere. Montane forest is believed to have survived in topographically complex areas that afforded an adequate degree of climatic

buffering (Fjeldsa, 1994; 1997a,b; Haffer, 1997; Fjeldsa, Lambin and Martens, 1999; DeKlerk *et al.*, 2002). On average, the lower altitude margins of these forests descended by up to 1,500m (Lovett, 1993). The additional modulating effects of favourable climate, soil and vegetation would have affected the precise location of these areas (Haffer, 1997; Fjeldsa, 1994; Tinsley *et al.*, 1996). Localized reduction in climatic variability would have favoured continuous forest survival throughout climatic vicissitudes of the Pleistocene (Fieldsa and Lovett, 1997b), providing long-term habitat refuge both for forest species and their component taxa (Lawes, 1990). These well-defined areas are referred to as ice-age refugia.

At the onset of warmer, wetter conditions characteristic of inter-glacials, the range of lowland forests will have expanded by gradual infiltration of floristic species, ultimately leading to the coalescence of previously isolated lowland forest-islands (Hamilton, 1988). Montane forests retreated again to higher altitudes.

ZOOGEOGRAPHY

Forest-bound taxa would inevitably have undergone a corresponding pattern of range expansion and contraction, retreating to refugia during glacial episodes, whilst expanding their ranges at rates corresponding to those of forest habitats during inter-glacials (Tinsley *et al.*, 1996; Fjeldsa and Lovett, 1997a; Fieldsa *et al.*, 1999). Likewise, the distribution of savanna-bound taxa will have undergone a reciprocal pattern of range expansion and contraction.

Given the apparent severity of the effects of recent climatic fluctuations on vegetation zones and the distribution of taxa occupying different habitats, it perhaps would be expected that the present distribution pattern should bear some form of physical imprint that reflects constraints imposed by the most recent glacial maximum (Diamond and Hamilton, 1980). Predictably, there is remarkable consistency in biogeographic pattern demonstrated by different groups, including butterflies, spiders, millipedes and other flightless insects, avifauna, amphibians and mammals (Wasser and Lovett, 1993; Seymour *et al.*, 2001). A trend emerges of common foci for peaks in species diversity and endemism in coastal and montane areas, whilst broad, spatially uniform ecotypes are more typically occupied by a relatively low number of taxa.

Though data are lacking for high-resolution analysis of distribution for most groups (Seymour *et al.*, 2001), the distribution of bird taxa is well known, much studied and has enabled an assessment of the significance of this distribution pattern. This group, whilst sharing the same general pattern as others studied, has shown in addition that species are broadly divisible into three categories, varying in their distribution pattern and separated on the basis of differences in taxonomic age and life-history strategies. The broad-scale consistency shared in biogeographic pattern between avifauna and other groups may, theoretically, extend to the fine-scale pattern shown by avifauna.

Avifauna is divided into taxonomically old, medium-old and young species (Fjeldsa, 1994). The oldest species, otherwise known either as paleoendemics (Lovett, 1993) or relicts (Flenley, 1979) have restricted ranges, and are generally found only in mountain refugia (DeKlerk *et al.*, 2002). It is probable that these taxa formerly occupied a much wider distribution range than at present (Hamilton, 1982), during a period when lowland habitats favoured their survival. They have since become isolated in montane habitats, and show a disjunct distribution.

Medium-old species, in contrast, have wide distributions and are today found in spatially uniform lowland forest areas that are characterized by a uniform pattern of climatic variability (Fjeldsa and Lovett, 1997b). These areas are specifically vulnerable to climatic fluctuations and are postulated to have undergone extreme habitat disturbance throughout the Pleistocene, a situation known to promote opportunism and genetic resilience (Fjeldsa and Lovett, 1997a). Historically, the ranges of taxa associated with lowland forest will have followed the distribution of this biotype, successively expanding and contracting, retreating to riparian habitats and other forested refugia (Fjeldsa, 1994). These refugia acted more as forest 'museums' than centers of speciation, as evidenced by the low numbers of young taxa in occupation there today (Ndjele, 1988 [vascular plants]; Colyn *et al.*, 1991 [primates]; Fjeldsa, 1994; Fjeldsa and Lovett, 1997b [avifauna]).

Taxonomically young species are further sub-divided into groups occupying montane refugia and groups that evolved at the periphery of lowland-forest blocks during glacial periods (Fjeldsa, 1994). The former group typically evolved in very well defined areas within topographically complex, mountain refugia and shows a highly restricted distribution range (Fjeldsa, 1994; Fjeldsa and Lovett, 1997c; Seymour *et al.*, 2001). These areas are today characterized by greater ecoclimatic stability than anywhere else on the continent, conditions that are believed to have prevailed locally, throughout the Pleistocene (Seymour *et al.*, 2001). As such, communities that evolved here will have minimum ability to adapt to conditions elsewhere, for which they have not been selected (Fjeldsa and Lovett, 1997a). Such low genetic resilience may have been accentuated by the high potential for genetic bottlenecking in refugia (Fjeldsa, 1994). Speciation for most taxa, under these conditions, is likely to have arisen through vicariance, though hybridization will also have been important in some groups. In contrast, groups postulated to have evolved at the periphery of forest blocks, in the transition zone between forest and savanna, arose under less predictable climatic conditions, and by definition, in a more dynamic landscape. Under these circumstances, they would have acquired a greater degree of genetic plasticity, as evidenced today by a more diffuse pattern of distribution as compared with mountain neo-endemics. Their propensity for migration makes it difficult to determine the precise location of these refugia. However, the range of possibilities is reduced by their apparent exclusion from lowland forest, probably by interspecific competition with better-adapted, 'medium-old' species. This competitive exclusion restricts neo-endemics to the periphery of lowland-forest blocks (Diamond and Hamilton, 1980).

RELATIVE IMPORTANCE OF HISTORIC CLIMATIC FLUCTUATIONS IN SHAPING PRESENT DISTRIBUTIONS

Tuomisto and Ruokolainen (1997) contested the importance of historic climate change in shaping the overall patterns of differentiation and distribution seen today and claimed that those predicted by the Pleistocene refuge hypothesis are the same as those expected as a result of present day environmental gradients. Present distribution patterns continue to reflect constraints imposed by climatic variability however, be these short or long-term fluctuations, and remain relevant to the interpretation of biogeographic patterns and evolutionary life-history strategies.

LOCATION OF ICE-AGE REFUGIA

Remarkable congruence exists between distribution data for many groups (Diamond and Hamilton, 1980). Locations of refugia are generally flagged not only by high levels of endemism but also by peaks in species richness, and as foci for species showing disjunct distributions (Fjeldsa and Lovett, 1997c; DeKlerk *et al.*, 2002). Taken together, these patterns have enabled the mapping of ice-age refugia, which have been located in Malawi/Tanganyika, the Angolan highlands, eastern Tanzania, Eastern DRC and adjacent mountains of the Albertine Rift, Cameroon, Sierra Leone, West Ghana and Guinea; there is also evidence of smaller refuges in Ethiopia (Bidgood and Friis, 1998; Largen, 2001; DeKlerk *et al.*, 2002) and the Congo basin (Fjeldsa, 1994; Fjeldsa and Lovett, 1997b; Colyn *et al.*, 1991; Tuomisto *et al.*, 1997).

EFFECT ON *XENOPUS*

Whilst broad division of the genus *Xenopus* is consistent with that of forest– non-forest biotypes, the distinctive patterns of distribution shown by constituent species of each of these groups is consistent with this pattern having been shaped by the most recent glacial episode. Not only do foci of species diversity and endemism fall in areas determined by previous studies to have been ice-age refugia, but also, lowlands are occupied by relatively few, widely distributed species. These data provide corroboration for results from previous distribution studies, which have shown evidence for contraction of forest-species ranges to refugia, and subsequent migration from these centres at varying rates, a process that has resulted in a pattern of increasing species poverty radiating from refugia (Carcasson, 1964 [butterflies]; Moreau, 1966 [avifauna]; Hamilton, 1982 [forest taxa])

Taxonomic age cannot easily be determined for *Xenopus* species, making it impossible to directly compare distribution data between this genus and avifauna. The difficulty in assessing age for *Xenopus* species is attributable to the inherent nature of their polyploid status; ascribing age to a

hybrid is confounded by that hybrid having inherited different chromosome sets from a number of different parent taxa during successive hybridization events. The determination, by a molecular clock technique, of time lapsed since divergence from a common stock refers to the original divergence event rather than time since the most recent hybridization event (Kobel and Du Pasquier, 1986; Kobel *et al.*, 1998). A species of high allopolyploid status however, is by definition the product of hybridization between two species whose combined chromosome complement amounts to the total in that hybrid. Since restricted range species living within postulated refugia are generally characterized by octoploid or dodecaploid chromosome constitutions, it may be inferred that they are younger than their lower ploidy counterparts occupying adjacent, more ecotypically uniform areas.

The distribution of high ploidy taxa is consistent with the theory that conditions of sympatry, as would have existed in refugia intermittently throughout the Pleistocene, promoted interspecific hybridization between species with formerly wider distributions. The benefits of hybridization and introgression for a species include the production of a range of recombinants that would, in theory, have increased the adaptive potential of that population (Werger, 1978), enabling the expansion of its range, following onset of more favourable conditions, into ranges previously occupied by each constituent parent taxon. The distribution of allopolypoid species with a higher chromosome number continues to be restricted to well-defined areas however, despite 14,000 years of forest expansion from refugia, perhaps owing to the fact that they are better adapted to areas that form the margins of ecological tolerance for parent taxa.

THESIS OUTLINE

Individual studies will focus on specific areas of biogeographical interest. However, given the inherent political and logistical problems restricting fieldwork in most of the biogeographically more appealing areas, it has not been possible to conduct studies on living specimens. Rather, an alternative source of material was required. Relative to many other anuran taxa, *Xenopus* is not very well represented in museum and university collections owing to its lifestyle and the necessarily unusual methods employed in its capture (Kobel *et al.*, 1996). Collections from specific localities however, are extensive and provide a rare opportunity for taxonomic investigation at the species level. Some of these collections were used as the basis of the series of taxonomic studies listed below.

Each study required taxa to be distinguished apart for the purpose of qualitative and quantitative investigations of phenetic similarity. However owing to museum specimens having been formalin-fixed and alcohol-stored, sometimes under sub-optimal conditions, the variety of techniques that might successfully be applied to distinguishing between taxa was necessarily limited. Of the range of species identification methods that have been applied to live or alcohol preserved specimens, most cannot easily be applied to formalin-fixed material. Comparative osteology however, provides

great potential in this respect. Despite osteology being the only method that allows the systematic position of fossil taxa to be ascertained, it is surprising that only a single study has utilized this method as a taxonomic tool for this genus (see Reumer, 1985), although other references have also been made to a range of specific osteological characters (e.g. Cannatella and Trueb, 1988a,b; Loumont and Kobel, 1991; Baez and Trueb, 1997; Fabrezi, 2001). Information provided by these studies provides a positive indication of the potential value of comparative osteology as a method for taxonomic investigation at the species level, and ultimately for application to the study of biogeography.

Whilst chapter 1 provides a general introduction to the genus *Xenopus* and its biogeography, chapter 2 provides an overview of osteology in the genus, and covers aspects common to all taxa examined during the course of this study, and is used as a framework upon which all subsequent descriptions are based. A number of misconceptions stemming from generalizations that appear in the literature are also addressed.

Chapter 3 assesses the effects of age, size and sex on the osteology of *X. l. laevis*, and serves to highlight a need for care when ascribing specific osteological attributes to a taxon without prior knowledge of the degree and form of intra-specific variation. The chapter also serves to identify specific osteological characteristics of *X. l. laevis* and provides the first full account of osteology for the taxon.

The osteology of four taxa whose ranges overlap in the vicinity of the central African highlands is examined in chapter 4. Tetraploids *X. fraseri* and *X. l. victorianus* each have wide distributions on either side of these highlands and octoploid inter-species hybrids *X. wittei* and *X. vestitus* are narrowly endemic. Osteology is examined for evidence that tetraploids may have been involved in the hybrid event that gave rise to these endemic octoploids. The osteology of each is described for the first time.

Xenopus l. bunyoniensis was widespread throughout lakes of southwestern Uganda in the first half of the last century, but has since been replaced in these lakes by three other taxa, *X. l. victorianus*, *X. wittei* and *X. vestitus*. Osteological evidence is sought in chapter 5 to clarify the taxonomic ambiguity surrounding whether *X. l. bunyoniensis* and *X. l. victorianus*, now common in these lakes, ought to be synonymized.

The osteology of a number of *X. muelleri* samples, collected from sites across their range, is examined in chapter 6 with a view to ascertaining whether the taxon is divisible into two discrete forms, an eastern and a western form, or whether their distributions form a polytypic cline. Material from the easternmost part of the *X. muelleri* range was collected during fieldwork in Swaziland and South Africa. Collections were made across a transect known to intersect a biogeographic boundary and area of sympatry between *X. l. laevis* and *X. muelleri*. Evidence is sought in chapter 7 from comparative osteology and a range of other techniques for hybridization between these two taxa.

Chapter 8 addresses the ambiguity that surround the phylogenetic position of *X. largeni*, an Ethiopian species. Its osteology is examined and compared with that of other *Xenopus* taxa with a view to identifying specific taxonomic affinities.

BIBLIOGRAPHY

Axelrod, D.I. and Raven, P.H. 1978. "Late Cretaceous and Tertiary vegetation history of Africa." Pp. 77-171 in Biogeography and Ecology of Southern Africa. Vol. 31, edited by Werger, M.J.A. The Hague: Junk.

Bàez, A.M. and Trueb, L. (1997). Redescription of the Paleogene *Shelania pascuali* from Patagonia and its bearing on the relationships of fossil and recent pipoid frogs. *Scientific papers, Natural History Museum, University of Kansas* **4**:1-41.

Bidgood, S. and Friis, I. (1998). *Sespania melanocaulis*, sp. nov. (Leguminosae subfam. Papilionoidea) from SW Ethiopia. *Nordic Journal of Botany* **18(2)**:209-213.

Bonnefille, R., and Riollet, G. (1988). The Kashiru pollen sequence (Burundi). Palaeoclimatic implications for the last 40,000yr B.P. in Tropical Africa. *Quaternary Research* **30**:19-35.

Bonnefille, R., Roeland, J.C. and Guiot, J. (1990). Temperature and rainfall estimates for the past 40,000 years in equatorial Africa. *Nature* **346**:347-349.

Boulenger, G.A. (1905). On a collection of batrachians and reptiles made in South Africa by C.H.B Grant, and presented to the British Museum by Mr. C.D. Rudd. *Proceedings of the Zoology Society, London* (**2**): 248-255.

Brown, D.S. (2001). Taxonomy, biogeography and phylogeny of the non-lacustrine African freshwater snails belonging to the genera *Ceratophallus* and *Afrogyrus* (Mollusca: Planorbidae). *Journal of Zoology, London* **255**:55-82.

Cannatella, D.C. and De Sá, R.O. (1993). *Xenopus laevis* as a model organism. *Systematic Biology* **42**:476-505.

Cannatella, D.C. and Trueb, L. (1988a). Evolution of pipoid frogs: inter-generic relationships of the aquatic frog family Pipidae (Anura). *Zoological Journal of the Linnean Society* **94**:1-38.

Cannatella, D.C. and Trueb, L. (1988b). Evolution of pipoid frogs: Morphology and phylogenetic relationships of *Pseudhymenochirus*. *Journal of Herpetology* **22 (4)**:439-456.

Carcasson, R.H. (1964). A preliminary survey of the zoogeography of African butterflies. *East Africa Wildlife journal* **2**:122-157.

Chesters, K.I.M. (1957). The Miocene flora of Rusinga Island, Lake Victoria, Kenya. *Paleontographica* **101B**:30-71.

Colyn, M., Gautierhion, A., Verheyen, W. (1991). A reappraisal of Palaeoenvironmental history in central Africa- evidence for a major fluvial refuge in the Zaire basin. *Journal of Biogeography* **18(4)**:403-407.

De Klerk, H.M., Crowe, T.M., Fjeldsa, J. and Burgess, N.D. (2002). Patterns of species richness and narrow endemism of terrestrial bird species in the Afrotropical region. *Journal of Zoology, London* **256**:327-342.

De Sa, R.O. and Hillis, D.M. (1990). Phylogenetic relationships of the pipid frogs *Xenopus* and *Silurana*: an integration of ribosomal DNA and morphology. *Molecular Biology and Evolution* **7(4)**:365-376.

Diamond, A.W. and Hamilton, A.C. (1980). The distribution of forest passerine birds and quaternary climatic change in Tropical Africa. *Journal of Zoology, London* **191**:379-402.

Fabrezi, M. (2001). A survey of prepollex and prehallux variation in anuran limbs. *Zoological Journal of the Linnean Society* **131**:227-248.

Fjeldsa, J. (1994). Geographical patterns for relict and young species of birds in Africa and South America and implications for conservation priorities. *Biodiversity and Conservation* **3**:207-226.

Fjeldsa, J., Lambin, E. and Martens, B. (1999). Correlation between endemism and local ecoclimatic stability documented by comparing Andean bird distributions and remotely sensed land surface data. *Ecography* **22(1)**:63-78.

Fjeldsa, J. and Lovett, J.C. (1997a). Biodiversity and environmental stability. *Biodiversity and Conservation* **6**:315-323.

Fjeldsa, J. and Lovett, J.C. (1997b). Geographical patterns of old and young species in African forest biota: the significance of specific montane areas as evolutionary resume. *Biodiversity and Conservation* **6**:325-346.

Flenley, J. 1979. The Equatorial Rainforest: A Geological History. London: Butterworths.

Gasse, F., Tehet, R., Durand, A., Gilbert, E. and Fontes, A. (1990). The arid humid transition in the Sahara and the Sahel during the last deglaciation. *Nature* **346 (6280)**:141-146.

Graf, J.-D. 1996. "Molecular approaches to the phylogeny of *Xenopus*". Pp. 379-388 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford. Clarendon Press.

Gurdon, J.B. 1996. "Introductory comments: *Xenopus* as a laboratory animal". Pp. 3-6 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford. Clarendon Press.

Haffer, J. (1997). Alternative models of vertebrate evolution in Amazonia: an overview. *Biodiversity and Conservation* **6**:451-476.

Hamilton, A.C. 1972. "The interpretation of pollen diagrams from highland Uganda." Pp. 46-149 in Palaeoecology of Africa, The Surrounding Islands and Antarctica. 1st ed., edited by van Zinderen Bakker Sr., E.M. Cape Town: Balkema.

Hamilton, A.C. ed. 1982. Environmental History of Africa: A Study of the Quaternary. London: Academic Press.

Hamilton, A. (1976). The significance of patterns of distribution shown by forest plants and animals in tropical Africa for the reconstruction of upper Pleistocene Palaeoenvironments: A Review. *Palaeoecology of Africa* **9**:63-97.

Hamilton, A. 1988. "Guenon evolution and forest history." Pp. 13-34 in A primate radiation: Evolutionary Biology of the African guenons. Ed. Gautier-Hion, A., Bourliere, F., Gautier, J.P. and Kingdon, J., Cambridge: Cambridge University Press.

Henrici, A.C. and Baez, A.M. (2001). First occurrence of *Xenopus* (Anura:Pipidae) on the Arabian peninsula: A new species from the upper Oligocene of Yemen. *Journal of Palaeontology* **75**(4):870-882.

Jolly, D., Taylor, D., Marchant, R., Hamilton, A., Bonnefille, R., Buchet, G. and Riollet, G. (1997). Vegetation dynamics in central Africa since 18,000yr BP: pollen records from the interlacustrine highlands of Burundi, Rwanda and western Uganda. *Journal of Biogeography* **24**(4):495-512.

Kobel, H.R., 1996. "Allopolyploid speciation." Pp391-400 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Kobel, H.R., Loumont, C and Tinsley, R.C. 1996. "The extant species." Pp. 9-33 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Kobel, H.R., Barandun, B. and Thiébaud, ChH. (1998). Mitochondrial rDNA phylogeny in *Xenopus*. *Herpetological Journal* **8** (**1**):13-18.

Kobel, H.R. and Du Pasquier, L. (1986). Genetics of polyploid *Xenopus*. *Trends in genetics* **2**:310-315.

Kobel, H.R., Du Pasquier, L., Fischberg, M. and Gloor, M. (1980). *Xenopus amieti* sp. nov. (Anura: Pipidae) from the Cameroons, another case of tetraploidy. *Revue Suisse Zoologie* **87** (**4**):919-926.

Largen, M.J. (2001). Catalogue of the amphibians of Ethiopia including a key for their identification. *Tropical Zoology* **14**(**2**):307-402.

Laurent, R.F. (1972). Amphibiens. Exploration Parc national des Virungas II. **Serial 22**:1-125.

Lawes, K.J. (1990). The distribution of the Samango monkey (*Cercopithecus mitis erythrarchus* Peters, 1852 and *Cercopithecus mitis labiatus* I. Geoffroy, 1843) and forest history in southern Africa. *Journal of biogeography* **17**:669-680.

Loumont, C. (1983). Deux espèces nouvelles de *Xenopus* du Cameroun (Amphibia, Pipidae). *Revue Suisse Zoologie* **90** (**1**):169-177.

Loumont, C. (1984). Current distribution of the genus *Xenopus* in Africa and future prospects. *Revue Suisse Zoologie* **91** (**3**):725-746.

Loumont, C. (1986). *Xenopus pygmaeus*, a new diploid pipid frog from rain forest of equatorial Africa. *Revue Suisse Zoology* **93**:755-764.

Loumont, C. and Kobel, H.R. (1991). *Xenopus longipes* sp. nov., a new polyploid pipid from western Cameroon. *Revue Suisse Zoologie* **98** (**4**):731-738.

Lovett, J.C. 1993. "Climatic history and forest distribution in eastern Africa." Pp. 23-29 in Biogeography and Ecology of the Rainforests of Eastern Africa, edited by Wasser, S.K. and Lovett, J.C. Cambridge: Cambridge University Press.

Marchant, R., Taylor, D. and Hamilton, A (1997). Late Pleistocene and Holocene history at Mubwindi swamp southwest Uganda. *Quaternary Research* **47**(**3**):316-328.

Moreau, R.E. 1966. The bird fauna of Africa and its islands. Academic press, New York and London.

Morrison, M.E.S. (1968). Vegetation and climate in the uplands of southwestern Uganda during the late Pleistocene period, 1. Muchoya Swamp, Kigezi district. *Journal of Ecology* **56**:363-384.

Ndjele, M. (1988). Principales distributions obtenues par l'analyse factorielle des resumes phytogéographiques resumes endémiques dans la flore du Zaire. *Monographs of Systematic Botany of the Missouri Botanical Gardens* **25**:631-638.

Ptacek, M.B., Gerhardt, H.C. and Sage, R.D. (1994). Speciation by polyploidy in treefrogs: multiple origins of the tetraploid, *Hyla versicolor. Evolution* **48(3)**:898-908.

Reumer, W.F. (1985). Some aspects of the cranial osteology and phylogeny of *Xenopus* (Anura,Pipidae). *Revue Suisse Zoologie* **92 (4)**:969-980.

Schmid, M. and Steinlein, C. (1991).Chromosome banding in Amphibia XVI. High resolution replication banding patterns in *Xenopus laevis. Chromosoma* **101(2)**:123-132.

Seymour, C.L., Klerk, H.M., Channing, A. and Crowe, T.M. (2001). The biogeography of the Anura of sub-equatorial Africa and the prioritization of areas for their conservation. *Biodiversity and Conservation* **10**:2040-2076.

Shackleton, N.J. and Kennet, J.P. (1975). Palaeotemperature history of the Cenozoic and initiation of Antarctic glaciation: oxygen and carbon isotope analysis in DSDP sites 277, 279 and 281. *Initial reports of the deep sea drilling project 29* **29**:743-755.

Street, A.F. 1981. Tropical palaeoenvironments. *Progress in human geography.* **5(1)**:157-179.

Tinsley, R. C. (1973). Studies on the ecology and systematics of a new species of clawed toad, the genus *Xenopus*, from western Uganda. *Journal of Zoology* **169**:1-27.

Tinsley, R. C. (1975). The morphology and distribution of *Xenopus vestitus* (Anura:Pipidae) in Central Africa. *Journal of Zoology, London* **175**:473-492.

Tinsley, R. C. (1981a). The evidence from parasite relationships for the evolutionary status of *Xenopus* (Anura: Pipidae). *Monitore Zoologica Italiano* **19**:367-385.

Tinsley, R. C. (1981b). Interactions between *Xenopus* species (Anura: Pipidae). *Italian Journal of Zoology* **9**:133-150.

Tinsley, R.C. (1995). A new species of *Xenopus* (Anura: Pipidae) from the highlands of Ethiopia. *Amphibia-Reptilia* **16**:375-388.

Tinsley, R.C. 1996. "Parasites of *Xenopus*." Pp. 233-251 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Tinsley, R.C. and Kobel, H.R., eds. 1996. The Biology of *Xenopus* 1st ed. Oxford: Clarendon Press.

Tinsley, R.C., Kobel, H.R. and Fischberg, M. (1979). The biology and systematics of a new species of *Xenopus* (Anura:Pipidae) from the highlands of Central Africa. *Journal of Zoology, London* **188**:69-102.

Tinsley, R.C., Loumont, C. and Kobel, H.R. 1996. "Geographical distribution and ecology". Pp. 35-59 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Tuomisto, H. and Ruokolainene, K. (1997). The role of ecological knowledge in explaining biogeography and biodiversity in Amazonia. *Biodiversity and Conservation* **6**:347-357.

Tymowska, J. and Fischberg, M. (1973). Chromosome complements of the genus *Xenopus*. *Chromosoma (Berlin)* **44**:335-342.

Wasser, S.K. and Lovett, J.C. 1993. "The Geological Evolution of East Africa." Pp. 30-47 in Biogeography and Ecology of the Rainforests of Eastern Africa. : Cambridge University Press.

Weissmen, A.I. and Coates C.W. (1944). Ovarian activity of *Xenopus laevis* while in captivity. *American Naturalist* **78**:383-384.

Werger, M.J.A. 1978. "The Karoo-Namib Region." Pp. 231-99 in Biogeography and Ecology of Southern Africa, edited by Werger, M.J.A. The Hague: The Hague:Junk.

Wilson, E.O. 1992. The diversity of life. Belknap Press, Harvard University Press.

Yemane, K., Bonnefille, R. and Faure, H. (1985). Palaeoclimatic and tectonic implications of Neogene microflora from the northwestern Ethiopian highlands. *Nature* **318**:653-656.

CHAPTER 2 : *XENOPUS* OSTEOLOGY

INTRODUCTION

The major divisions within Anura are based primarily on variation in osteology. Furthermore, osteology provides the only means by which the taxonomic position of fossil Anura can be determined (Trueb, 1973). For over a century, since Cope's first classification of frogs (1864) systematists have relied on only a limited number of characters, many osteological, their taxonomic significance, distribution and degree of variation have been little understood (Trueb, 1973; Lynch, 1973). These classifications have drawn on fundamental differences in pectoral structure (Cope, 1964; Griffiths, 1963), maxillary dentition, centra arrangement (Nicholls, 1916; Reig, 1958; Tihen, 1965), thigh musculature (Noble, 1922, 1931) and differences in larval morphology (Orton, 1953, 1957). No consensus was reached however, until quantitative techniques were applied. Kluge and Farris (1969) provided the first osteology-based index of phyletic distance between anuran groups. Their phylogeny was similar to modern phylogenies which use a multitude of characters, relating not only to osteology, but also to musculature, gross morphology, behaviour and larval characters and utilize powerful methodology including quantitative phyletics, character weighting and computer generated phylogenies (Lynch, 1969; Lynch 1973; Cannatella, 1985; Cannatella and Trueb, 1988a,b; Cannatella and De Sa, 1993; Trueb, 1993, 1996).

Xenopus phylogeny

Pipidae are considered to be among the more primitive clades of Anura, and the genus *Xenopus* primitive among pipids. The distribution of extant pipid taxa extends across two continents, being represented in South America by *Pipa* and in Africa by *Hymenochirus, Pseudhymenochirus, Xenopus* and *Silurana*.

Phylogeny of the Salientia (Anura)

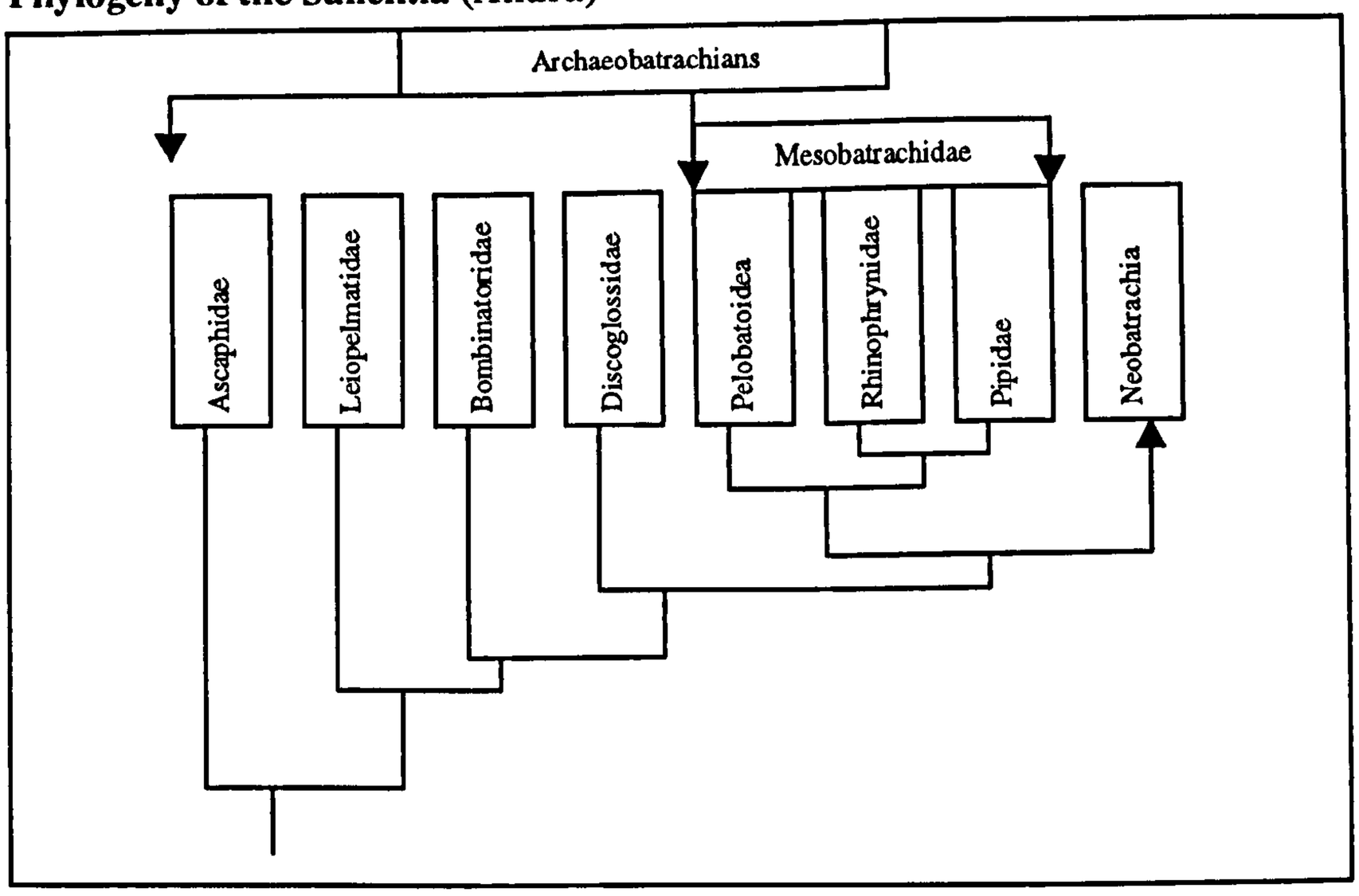


Figure 2-1

Phylogenetic position of Pipidae within the Anura. Neobatrachians, or modern Anura, include Leptodactylidae, Bufonidae, Hylidae, Sooglossidae, Microhylidae, Hyperolidae, Ranidae, Rhacophoridae, Arthroleptidae and Dendrobatidae (modified from Trueb, 1996).

Phylogeny of Pipidae

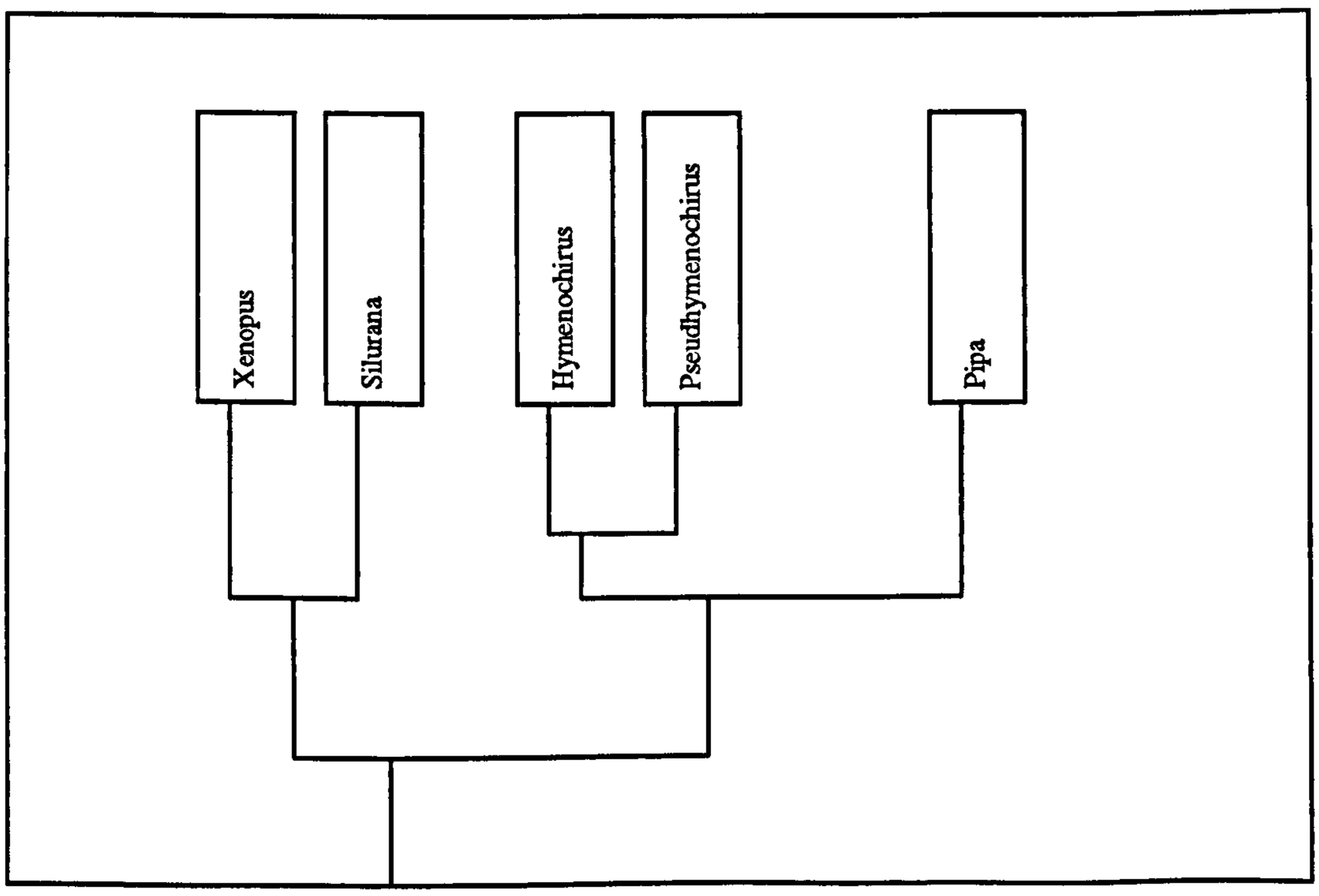


Figure 2-2

Phylogenetic position of *Xenopus* within Pipidae (modified from Trueb, 1993, 1996).

Palaeontology

Pipids are relatively well represented in the fossil record, given the paucity of anuran fossils in general (Estes, 1975b; Baez, 1996) and date from the Lower Cretaceous, 120MyrBP, of Israel. There is a considerable palaeontological literature describing fossil Pipidae from sites as far apart as the Yemen (Upper Oligocene, 26MyrBP), South America (various dating from the Upper Cretaceous, 100-95MyrBP, of Argentina) and across Africa (also dating from the Upper Cretaceous, 90-80MyrBP) and many more describing extant pipid forms (Paterson, 1939, 1945, 1955; Nevo, 1956; Sedra and Michael, 1957; Reig, 1959; Bustos, Caprini, Kraglievich and Corro, 1960; Vergnaud-Grazzini, 1966; Estes, 1975a,b, 1977; Baez, 1981; Baez, 1996; Baez and Trueb, 1997; Baez and Pugener, 1998; Baez and Rage, 1998; Henrici, 1998; Trueb, 1999; Baez, Trueb and Calvo, 2000; Trueb, Pugener and Maglia, 2000; Trueb and Massemin, 2000; Henrici and Baez, 2001). The record for *Xenopus* however, is not so extensive. Furthermore, of the fossils identified as *Xenopus*, only a few may still be valid (Baez, 1996; Henrici and Baez, 2001), the remainder being assigned instead to *Silurana* or to other pipids. Other fossils, which have tentatively been assigned to *Xenopus*, are juvenile in form, making the precise determination of their phylogenetic position problematic, especially since the effects of age on osteology in *Xenopus* are unknown. In her review of pipid fossils, Baez (1996) considered that perhaps only a single record should retain its *Xenopus* status, a record based on a specimen which has since been lost (*X. stromeri* Ahl, 1926 dating from the Miocene, 20MyrBP, of southwestern Africa). A second un-refuted *Xenopus* fossil, *X. arabiensis*, has since been described from the Yemen (Henrici and Baez, 2001; see above). Though somewhat debatable, existing palaeontological evidence appears to indicate that the distribution range of *Xenopus* (including putative *Silurana* species) at the time Gondwanaland split, between 90 and 110MyrBP, extended from Yemen to In Beceten, Niger in Africa (un-named, Late Cretaceous, 100-95MyrBP) to northwestern Patagonia in South America (un-named, Late Cretaceous, 90-80MyrBP).

Ambiguity surrounding the taxonomic determination of fossils indicates a need for an overview of osteology in extant *Xenopus* species. Such a study would serve to clarify the systematic position of a number of *Xenopus*-like fossil taxa (see Baez, 1996), and would enable a reassessment of the historic distribution range of the genus. An appropriate starting point for any such study would be an examination of the degree of intra-specific variation. This would place into systematic context and provide the necessary perspective for a study of the form and degree of inter-specific variation. Whilst a number of studies into the phylogeny of the genus have examined a range of *Xenopus* species, none has referred specifically to inter- or intra-specific variation and all have relied on only a small selection of osteological characters (Reumer, 1985; Loumont and Kobel, 1991; Trueb and Hanken, 1992; Henrici and Baez, 2001). Furthermore, many of these studies have been based on only a small number of specimens of each taxon, samples often being of mixed sex composition and including juveniles. It would appear from the current study and from results presented in following

chapters, that these samples have represented too little of the natural variation that exists within the genus. As a consequence, many generalizations regarding the osteology of the genus have become commonplace in the literature, some of which may conceal taxonomically significant osteological variation. This explains, at least in part, why, in Baez's review of pipid fossils (1996), she questioned the identity of many of those classified as belonging to this genus. Until the systematic importance of a number of these misconceptions is addressed, the significance of their continued incorporation into osteology-based phylogenies cannot fully be appreciated.

AIM

The following account will describe the osteology of adult *Xenopus* specimens, and is based on observations made of a range of taxa (see below) and on information from the literature regarding additional taxa not examined during the course of the present study (specifically, *X. longipes*). Where aspects of osteology are highly intra- or inter-specifically variable, this will be noted and the range of observed variation described. In the event of a single taxon varying significantly from all others with respect to any specific character, the taxon name and character state will be given. For detailed descriptions however, the reader is referred to individual species accounts in following chapters. Commonly held misconceptions and contradictions occurring in the literature will be addressed, and their taxonomic significance assessed. The account is intended as a basic framework upon which subsequent species accounts will be based.

MATERIALS AND METHODS

The following description is based on observations made of over 230 adult specimens, representing 12 taxa collected from localities across sub-Saharan Africa: *X. l. laevis, X. l. sudanensis, X. l. victorianus, X. l. bunyoniensis, X. gilli, X. fraseri, X. wittei, X. vestitus, X. muelleri* (east and west), *X. borealis, X. largeni*; X-rays were also obtained of *X. clivii* (localities listed in individual accounts). Specimens were skinned, eviscerated and cleared for osteological examination; the clearing procedure was modified from methods used by Wassersug (1976). Tissue was softened in 0.4% KOH for periods ranging from 1 day to over a week (a period determined by the rate of tissue softening) at a temperature of 25°C under UV light, the solution changed daily. Specimens were subsequently alizarin-stained and stored in 70% glycerol, a thymol crystal added to prevent fungal growth. Following removal of muscle tissue using fine-tipped forceps, specimens were examined using a Leica binocular microscope under fibre-optic illumination.

RESULTS

Osteology of the genus *Xenopus*

Cranium

Frontoparietal

The frontoparietal (Illustration 2-1) is the main anterodorsal component of the braincase and is azygous. It is parallel-sided or hourglass-shaped with a modestly curved or square posterior margin, and an obtusely (occasionally acute) angled anterior margin that extends to partially overlap the nasal. The element widens at its point of association with occipitopetrosals. Parasagittal crests delineate the skull table, which extends caudally from anterolateral corners of the element, meeting just short of the posterior margin and are present in all mature specimens. The orientation of crests varies inter-specifically, occurring medially as a single crest, laterally as separate parallel ridges or diverging to form straight or sigmoid margins to the skull table. Some degree of intra-specific variation in the orientation of these crests characterizes *X. l. laevis* taxa, in which the skull table varies from being rectangular in some specimens to forming a pronounced sagittal ridge in others. Approximately two thirds of the element lies anterior to its point of lateral association with occipitopetrosals, the remainder is bordered by this element. Laterally, the element may be fused or unfused to the sphenethmoid. In transverse profile, the element is flat at its far rostral and caudal extremes, whilst being curved medially. The pineal foramen occurs within the anterior half of the element and is present in all specimens, though its relative position is inter-specifically variable.

Sphenethmoid and vomer

The sphenethmoid (Illustration 2-2) associates laterally with the frontoparietal and posteriorly with occipitopetrosals, forming the anteroventral component encasing the brain. Though generally rectangular in ventral profile, the sphenethmoid may be waisted and is evenly curved in transverse profile along most of its length, becoming flat towards its site of association with occipitopetrosals. Here it forms both anterior and ventral margins to prootic foramina before narrowing into a spatulate, caudal process that is positioned ventral to the inter-occipitopetrosal gap or suture, extending almost to the base of the cranium. This caudal process may be either parallel-sided or rhomboid in shape.

A narrow cultriform process extends medially from the anterior margin of the sphenethmoid, reaching as far as the pars dentalis (dental row) of premaxillaries. The tapered tip of this process rests on the dorsal surface of premaxillaries. The vomer is positioned ventral to the sphenethmoid at the level where cultriform process and main sphenethmoid body meet. In all species with the exception of *X. muelleri* (west), this element is edentous. Whilst usually azygous, the vomer may also be paired or fractured into multiple fragments and is highly variable in shape. In all species except *X. largeni*, and

occasionally *X. fraseri* and *X. wittei*, orbitonasal foramina are not enclosed by bone, represented instead as a pair of laterally-displaced indentations along the anterior margin of the element.

Size and relative position of optic foramina are highly variable. They range in size from minute to a quarter the length of the main sphenethmoid body and may be positioned laterally or ventromedially on the sphenethmoid. Retractor bulbi muscle scars (rtbs) extend medially from the anterior margin of these foramina. The gap separating scars ranges from being wide (*X. fraseri* and *X. muelleri* west) to being narrow, occasionally forming a continuous trough across the element (*X. l. laevis*).

Occipitopetrosal

Prootics and exoccipitals are indistinguishably fused in all specimens and shall be referred to throughout this thesis as occipitopetrosals (Illustration 2-1, Illustration 2-2). These paired elements form the caudal base of the braincase, associating dorsally with the frontoparietal, ventrally with the sphenethmoid and medially with one another around the foramen magnum. Anterior margins may be either perpendicular or posterolaterally oriented with respect to the main body axis, and posterior margins posterolaterally oriented, converging laterally on cristae parotica, which vary from being short in *X. fraseri* and *X. wittei* to long in *X. laevis* and *X. muelleri* (east). Occipitopetrosals form the posterior margin to prootic foramina. The foramen magnum varies in size, has evenly curved dorsal (inverted V-shape in *X. vestitus*) and ventral margins and bears bilateral, reniform surfaces for articulation with the atlas.

The degree of dorsal occipitopetrosal superstructure is variable. A prominent reniform epiotic eminence, which curves medially around the auditory capsules characterizes some taxa (*X. fraseri* and *X. wittei*), whilst in others (*X. muelleri* and *X. laevis*), the anteromedial portion of this eminence is absent and posterolateral portion accentuated to form a pronounced, straight crest which is aligned with posterolateral alae of the frontoparietal (Illustration 2-1), and extends diagonally across the auditory capsule to the medial margin of the pars interna plectri. Generally, the wider the element, the more concave is its dorsal surface and the better developed this diagonal ridge. Paired jugular foramina flank the foramen magnum and are divided internally by a bony septum that separates medially-positioned jugular foramina from laterally-positioned inferior perilymphatic foramina. Ventrally, the element bears paired Eustachian troughs that curve posteromedially, circumventing auditory capsules. Pterygoid knobs (*sensu* Baez and Trueb, 1997) border troughs anteriorly, articulating with the sphenethmoid, whilst, posteriorly, troughs are bordered by ridges that vary in prominence and provide sites of attachment for ligaments that support otic plates. A pair of bony protrusions extends posteriorly from midway along these ridges, providing an attachment surface for cervical muscles. These protuberances vary in size, ranging from inconspicuous bulges in some to long, narrow, posterolaterally-directed processes in others. Posterior to ridges, the element assumes a

convex structure, the posterior margin of which is interrupted by the fenestra ovalis, an elongate socket that accommodates the pars interna plectri of the columella.

Pterygoid

The tri-radiate pterygoid (Illustration 2-1, Illustration 2-2) is anchored at its base to the pterygoid knob of occipitopetrosals. From here, rami extend in anterior, lateral and medial directions: anterior rami extend to form the lateral orbit margin, and are divided into proximal and distal flanges. The former are deep, ventromedially-oriented and straight, extending alterolaterally. The latter are narrow, dorsomedially-oriented and are convex with respect to one another. They associate with the dorsal face of maxillaries by means of a sliding cartilaginous articulation and curve medially towards the planum antorbitale. Lateral rami are narrow, oriented posteroventrally and extend around the quadrate/ pars articularis of occipitopetrosals. Medial rami, or otic plates, extend medially to form a thin plate-like floor to the Eustachian trough. In all but one species (*X. largeni*), the otic plate is bifid; one short, tapered ala extends anterolaterally towards the sphenethmoid, whilst the other, larger ala, extends medially (in *X. muelleri*, alae are approximately equal in size). The distance between medial margins, or the inter-otic gap, is inter-specifically variable.

Tympanosquamosal and columella

The tympanosquamosal (Illustration 2-1, Illustration 2-2) is a compound element formed by fusion between tympanic annulus and squamosal. The former articulates at its base with the crista parotica, and forms an anterolaterally directed, round to sub-oval, conch-like annulus, cleft along its posterior margin to allow passage of the long, narrow columella, or stapes. In some species, the cleft may be modified into otic processes that extend almost all the way around the shaft, or pars media plectri, of the columella. The annulus base, in some species, forms a dorsally cleft stem that holds the element away from cristae parotica, whilst in others this stem is missing, the annulus appearing to rest flush on cristae parotica.

The columella curves anteromedially, clinging to the profile of the skull and pivoting at its point of articulation with the annulus. At its base, it is anchored by the pars interna plectra in the fenestra ovalis. Relative lengths of the columella, anterior and posterior from its pivot point are variable, and depend on the length of the crista parotica.

The squamosal is represented by a narrow arm that extends anteriorly from the dorsal rim of the tympanum to articulate either with the dorsal or posterior face of the anterior pterygoid ramus by means of a zygomatic ramus, which may be expanded.

Maxillary Arch

These paired, toothed elements form an arch that extends between a quarter and over half way across the orbit and may be either square (*X. muelleri*) or tapered distally (Illustration 2-1, Illustration 2-2). Premaxillaries measure between a third and one fifth the length of maxillaries and

are less inter-specifically variable than the latter in relative length. Maxillary elements are roughly triangular in cross section, the dental row (ventral), palatine (medial), and facial (lateral) ridges extending radially from the main axis of the element. The dental ridge extends along the entire length of premaxillaries and along 50-90% the length of maxillaries. The total number of small, monocuspid, pedicellate teeth is variable. Palatine processes of premaxillaries form a deep shelf whose oblique posterolateral margin extends to overlap those of the maxillary. The palatine shelf is thin, widest at the junction between premaxillaries, gradually narrowing along the length of the arch. The pars facialis of maxillaries is robust, extending only along the portion of maxillary arch that is anterior to the orbit, occasionally overlapping with alary processes anteromedially. A pair of alary processes extends dorsally from the anteromedial region of premaxillaries.

Mandible

The lower jaw comprises paired dentaries and angulosplenals, the latter are generally (with the exception of *X. largeni*) evenly curved with a well-developed dosolaterally-projecting coronoid flange in the posterior region, whilst narrowing towards the mandibular symphysis (Illustration 2-3). Dentaries extend to meet at the mandibular symphysis, extending alongside angulosplenals for most of their length.

Nasal and septomaxillary

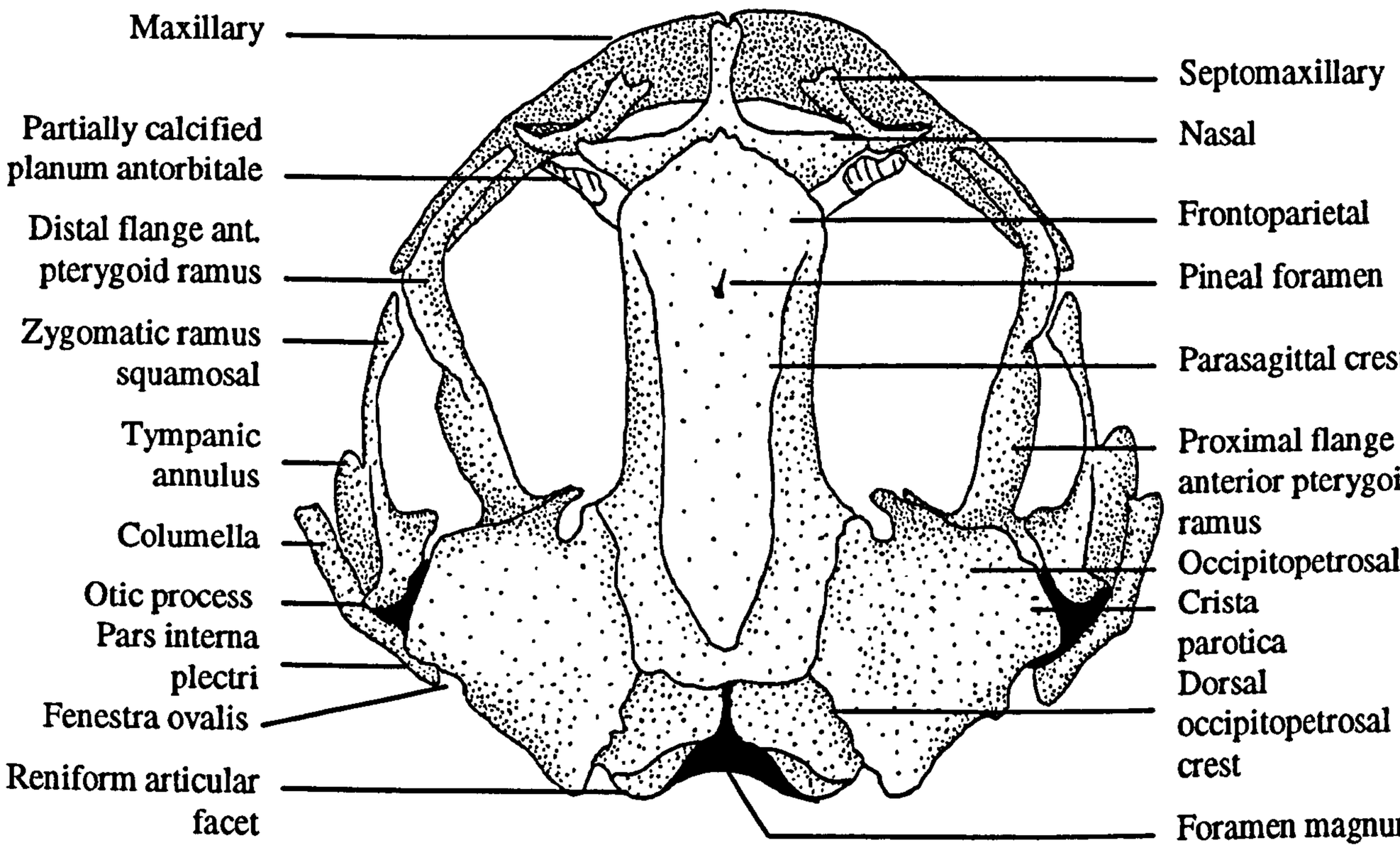
The tri-radiate nasal (Illustration 2-1, Illustration 2-2) is azygous in all but one species (*X. longipes* in which it is paired [Loumont and Kobel, 1991]). The rostral extension reaches the anterior margin of the skull and may be medially scarred and/or rostrally cleft and either parallel-sided or bulbous. Paired semi-lunar shaped, lateral extensions are overlapped posteriorly by the anterior frontoparietal margin, and extend beyond anterolateral corners of this cranial element. Small, bilateral processes protrude from the anterior margin of nasals in the most convex part of the element in some taxa. Paired septomaxillaries are sickle-shaped dermal elements that are embedded within nasal cartilages. In some species, each is pierced by a foramen, the lacrimonasal duct through which sub-ocular tentacles pass. These may appear instead as indentations along either the dorsal or ventral surface of the element. In other cases evidence of these ducts is altogether absent.

Calcification of cartilage elements

Calcification of rostrally-positioned cartilage bodies (Illustration 2-1, Illustration 2-2) is common in most species, however the prevalence and sequence in which calcification occurs varies inter-specifically. The following cartilage may become calcified; planum antorbitale, element A (*sensu* Smirnov, 1994), septum nasi, alary cartilage (nariale, of Bernasconi, 1951) and cartilage supporting the anterior ramus of the nasal.

Fusion between certain elements

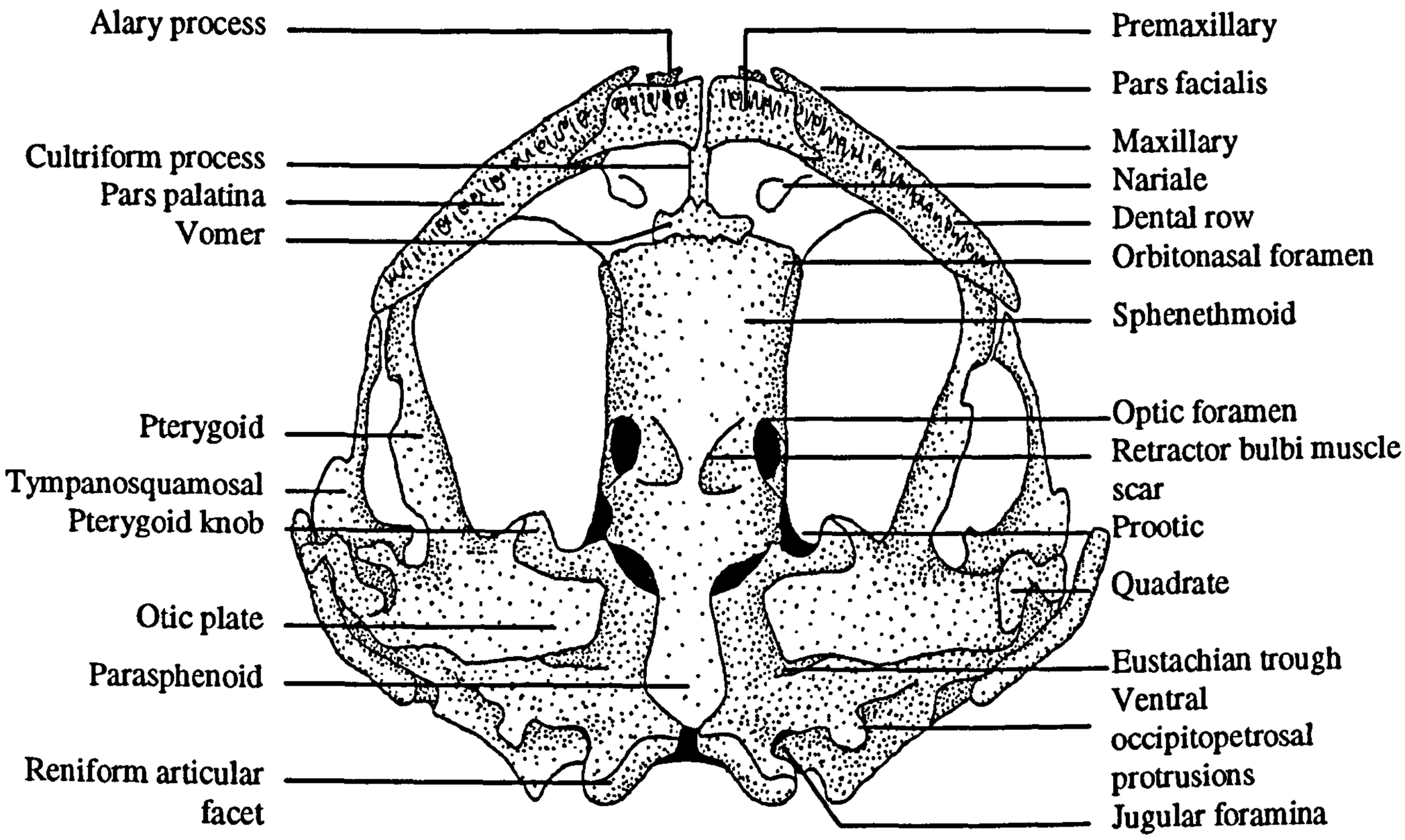
The degree of fusion between cranial elements (Illustration 2-1, Illustration 2-2) varies intra-specifically; in some specimens, lateral fusion between frontoparietal and sphenethmoid may be complete, and each in turn fused to occipitopetrosals, which themselves might be medially fused to enclose the foramen magnum. In others, cranial elements remain distinct. Some species exhibit a certain propensity for fusion of these elements (especially *X. fraseri* and *X. wittei*), whilst in others fusion rarely occurs. Similarly, there is a considerable degree of intra-specific variation in the extent of calcification of a number of rostral cartilaginous elements. The sequence of calcification varies inter-specifically, certain elements remaining uncalcified in some species.



Dorsal view *Xenopus laevis laevis* skull

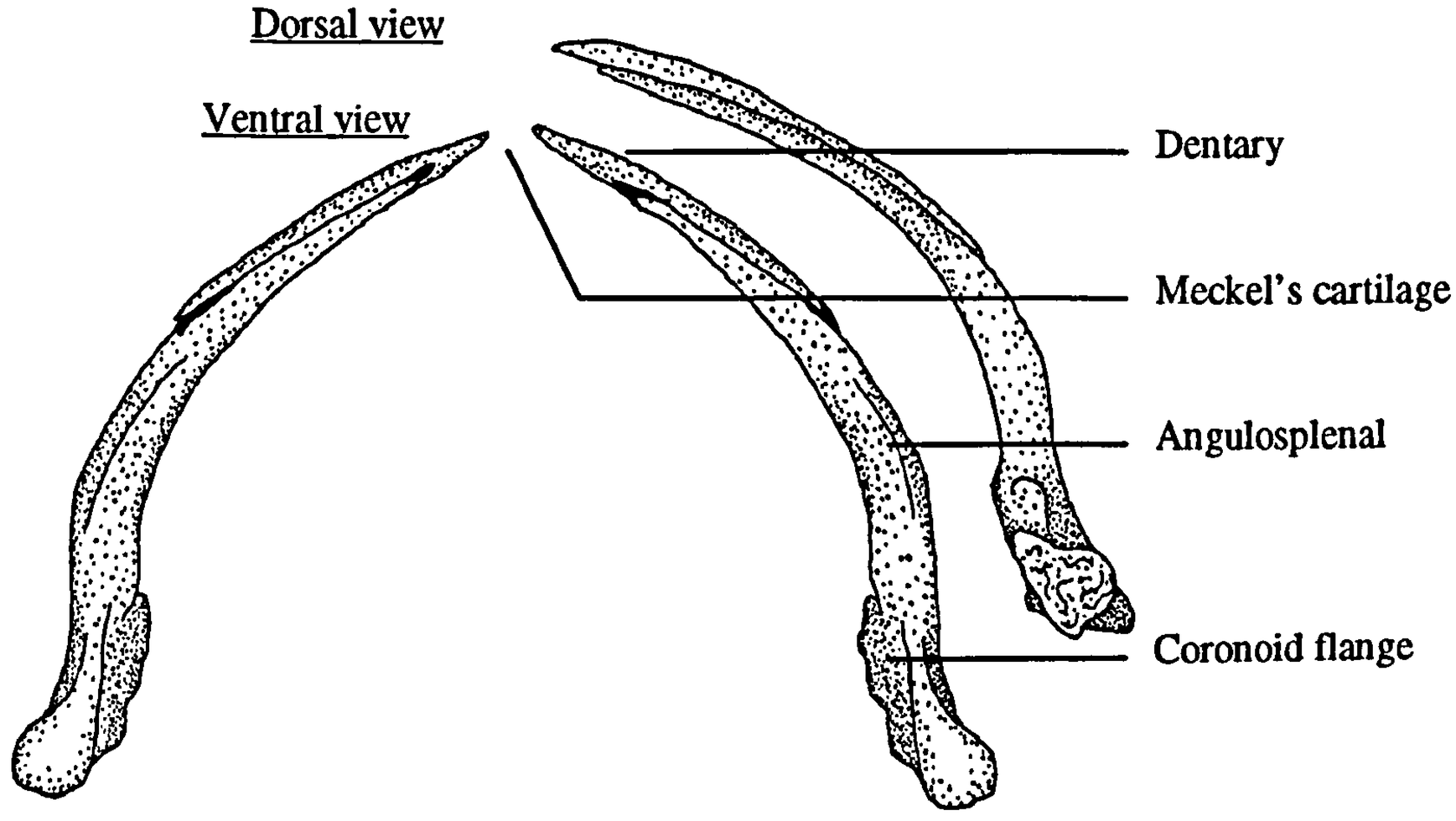
Illustration 2-1

(Maxillary and pre-maxillary not distinguished apart here for clarity.)



Ventral view *Xenopus laevis laevis* skull

Illustration 2-2



Xenopus laevis laevis mandible

Illustration 2-3

Hyolaryngeal apparatus

In females, the ossified component of the larynx consists of paired posteromedial processes. These are long, straight, narrow elements that bear posterior, cartilaginous epiphyses and are integrated into a cartilaginous complex that forms the laryngeal body (described by Sasson and Kelley, 1986; Trueb, 1996). Beyond size, very little inter-specific variation exists between these elements in females. In mature males, posteromedial processes are sigmoid in shape and are integrated into an extensive area of calcification (Illustration 2-4). In some males, cartilaginous elements may calcify extensively. This pattern of calcification varies inter-specifically.

Axial skeleton

The vertebral column (Illustration 2-5, Illustration 2-6) comprises eight pre-sacral vertebrae, a compound element formed from modification of the IXth vertebra — the sacrum — and the urostyle. The urostyle is a compound element in itself, the product of fusion between at least two vertebrae, as evidenced by the presence of two pairs of spinal foramina. Vertebrae increase in length towards the sacrum. Each pre-sacral vertebra is dorsoventrally compressed, and all except the atlas bears paired transverse processes. Pre-sacral vertebrae are non-imbricate and the degree of dorsal ornamentation variable. In cases of extreme ornamentation, a series of deep ridges and sulci adorn the dorsal surface of neural arches. Generally a single dorsal or neural spine develops on each vertebra, though double spines may develop either on the atlas alone (*X. laevis, X. borealis, X. muelleri* west) or on all pre-sacrals (*X. muelleri* east). All vertebrae except for the atlas bear post-zygapophyses, and all except atlas and axis possess pre-zygapophyses. These are longitudinally sliding, articulating fluted clasps, positioned laterally on neural arches, the pre-zygapophyses forming the outer clasp. Like vertebrae, they too increase in length towards the sacrum. Positioned anterior to the base of transverse processes on pre-sacrals II-IV and adjacent to the base of transverse processes on vertebrae V-VIII, their superstructure is not always symmetrical and may vary along the length of the column. Ventrally, adjacent neural arches articulate intimately and are opisthocoelous.

Atlas

The atlas (Illustration 2-5, Illustration 2-6) is robust, bearing laterally-displaced atlantal cotyles that articulate anteriorly with the foramen magnum. Dorsally, the anterior atlas margin may be straight or cotyles may be separated by a shallowly concave anterior margin, and ventrally by a convex margin, which in most cases is medially notched. The atlas is widest at its anterior margin and generally bears a single posteriorly-directed, dorsal, spinous process. In smaller species, the posterior margin is often straight.

Transverse processes

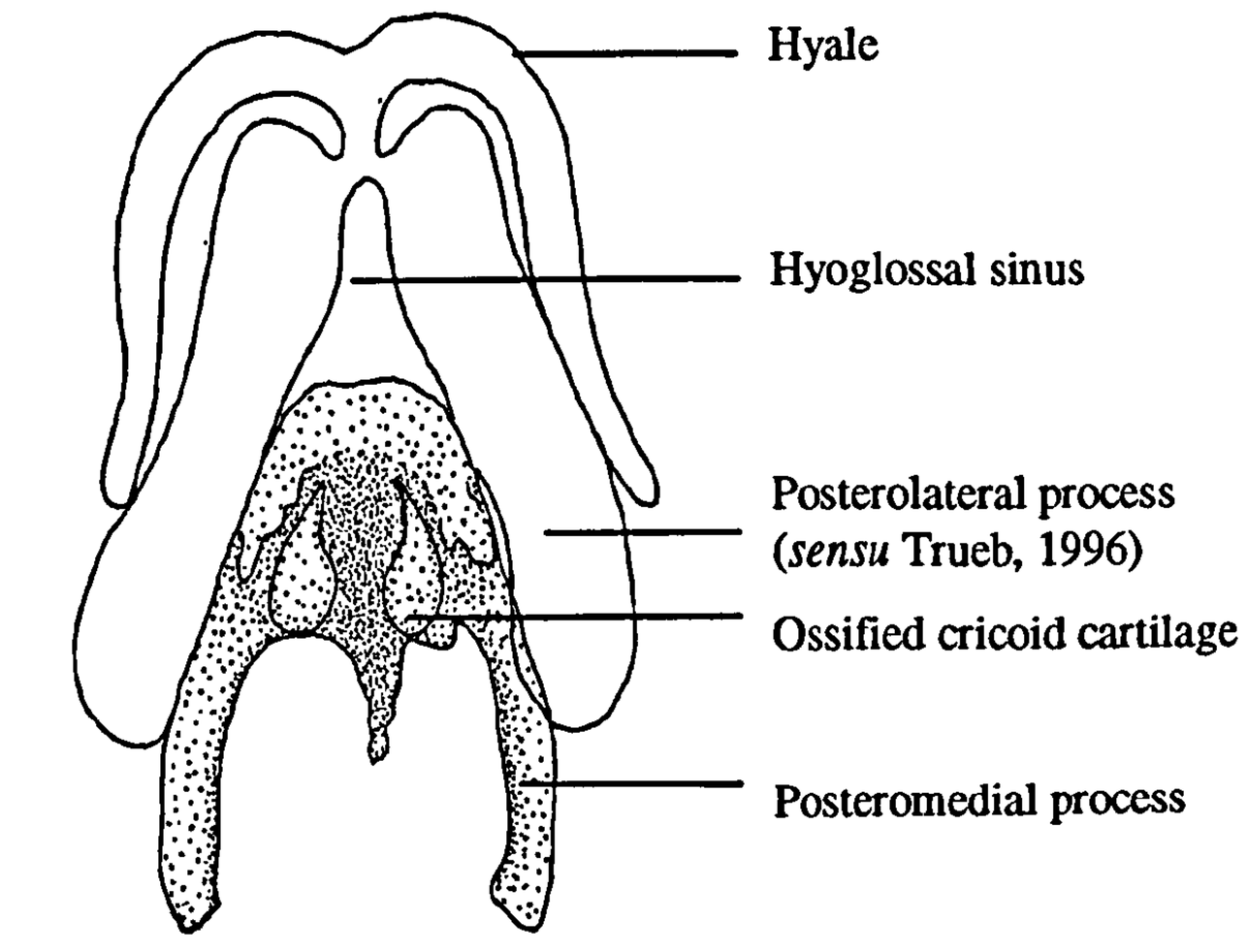
Transverse processes (Illustration 2-5, Illustration 2-6) arise from pedicels that extend laterally from each vertebra, each separated from the next by a pair of large spinal foramina.

Processes extending from pre-sacral II are short and robust, are widest at their mid-point and are anterolaterally-orientated on leaving the vertebra, arching posterolaterally. Transverse processes on pre-sacral III are generally the longest of all transverse processes. They are posterolaterally or perpendicularly orientated on leaving the vertebra, curving posteroventrally. Transverse processes on pre-sacral IV are generally slightly shorter and more strongly curved than the anteriorly adjacent pair, curvature being more on a horizontal plane. Transverse processes of the remaining four vertebrae are anterolaterally orientated, straight, narrow and tapered, increasing in length and acuteness (angle with respect to the zonal plane) towards the sacrum.

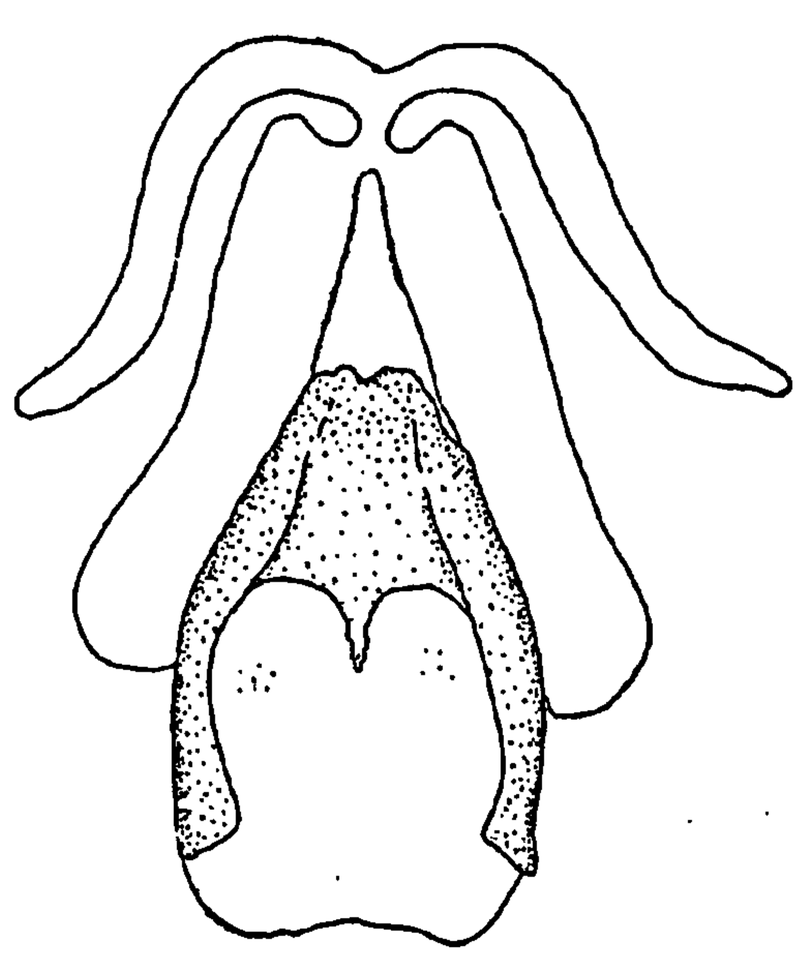
Sacrum and Urostyle

The VIIIth vertebra articulates, by means of post-zygapophyses, with pre-zygapophyses of the sacrum (Illustration 2-5, Illustration 2-6). This paired bi-laterally triangular structure is usually parallel-sided but lateral margins may occasionally either converge or diverge anteriorly. This structure facilitates a sliding articulation with iliac processes of the pelvic girdle during fore-aft movement, a characteristic feature of the genus. In some specimens, the element is symmetrical around the transverse plane, whilst in others the element is greatly extended in a posterior direction.

The greatly elongated urostyle is fused to the sacrum, which when the animal is maximally contracted, will reach as far as the ischium of the pelvic girdle. The urostyle bears two pairs of spinal foramina, the proximal pair considerably larger than the distal pair, and is ventrally keeled proximally, becoming round in cross section distally, the keel extending along approximately 75% of the element.



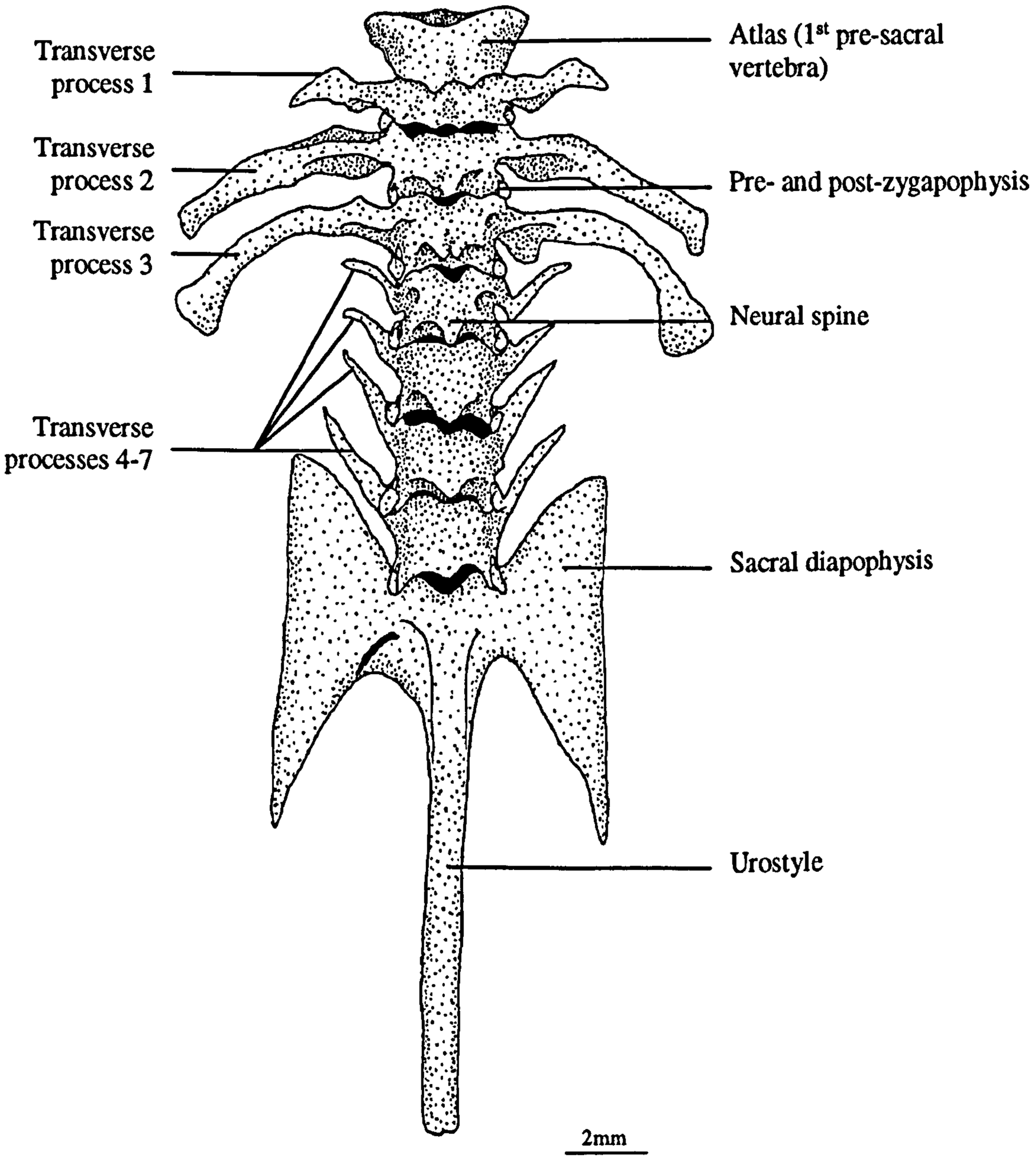
Dorsal view *Xenopus laevis laevis*
larynx (male)



Ventral view *Xenopus laevis laevis*
larynx (male)

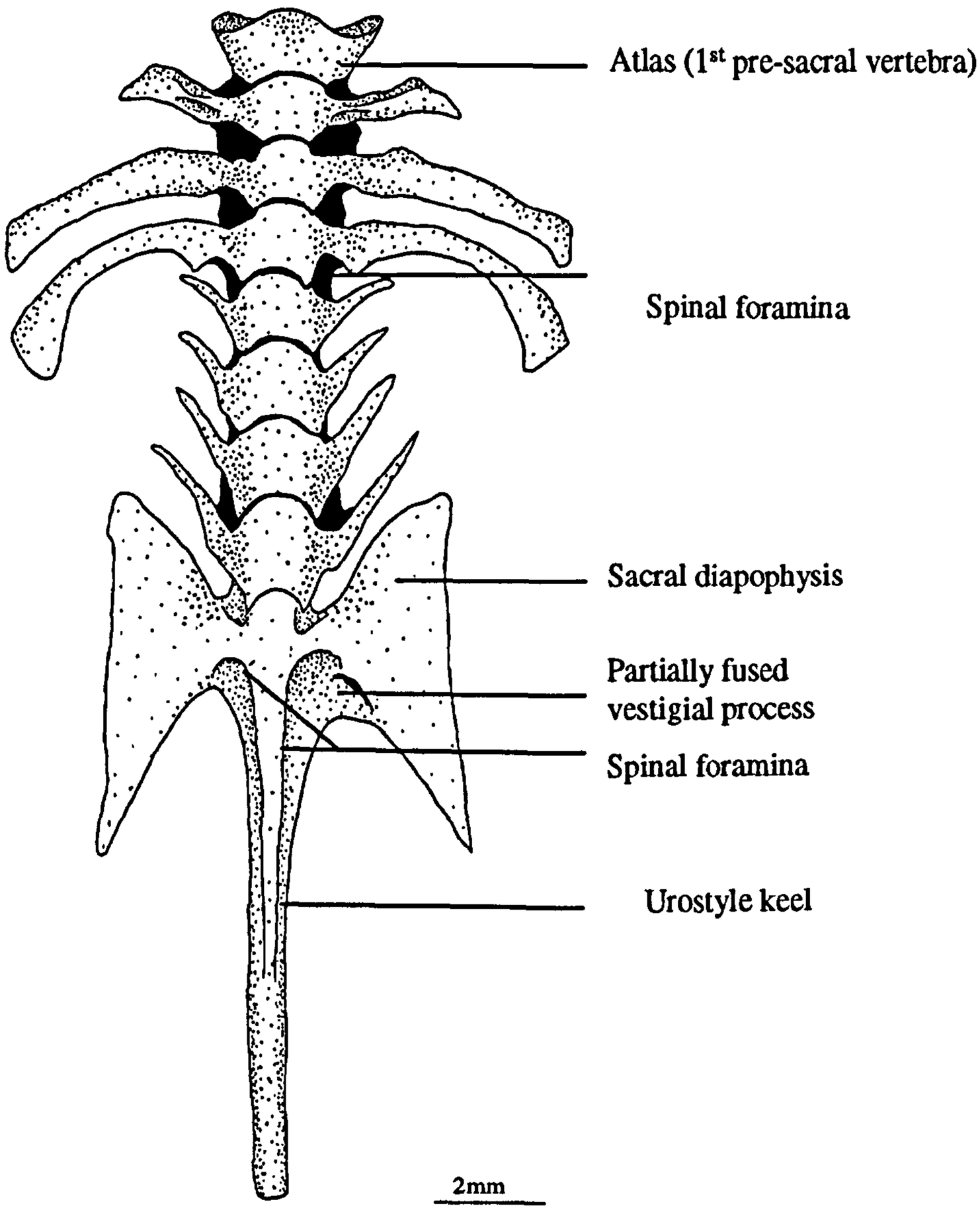
2mm

Illustration 2-4



Dorsal view *Xenopus laevis laevis* vertebral column

Illustration 2-5



Ventral view *Xenopus laevis laevis* vertebral column

Illustration 2-6

Anterior appendicular skeleton

Pectoral girdle

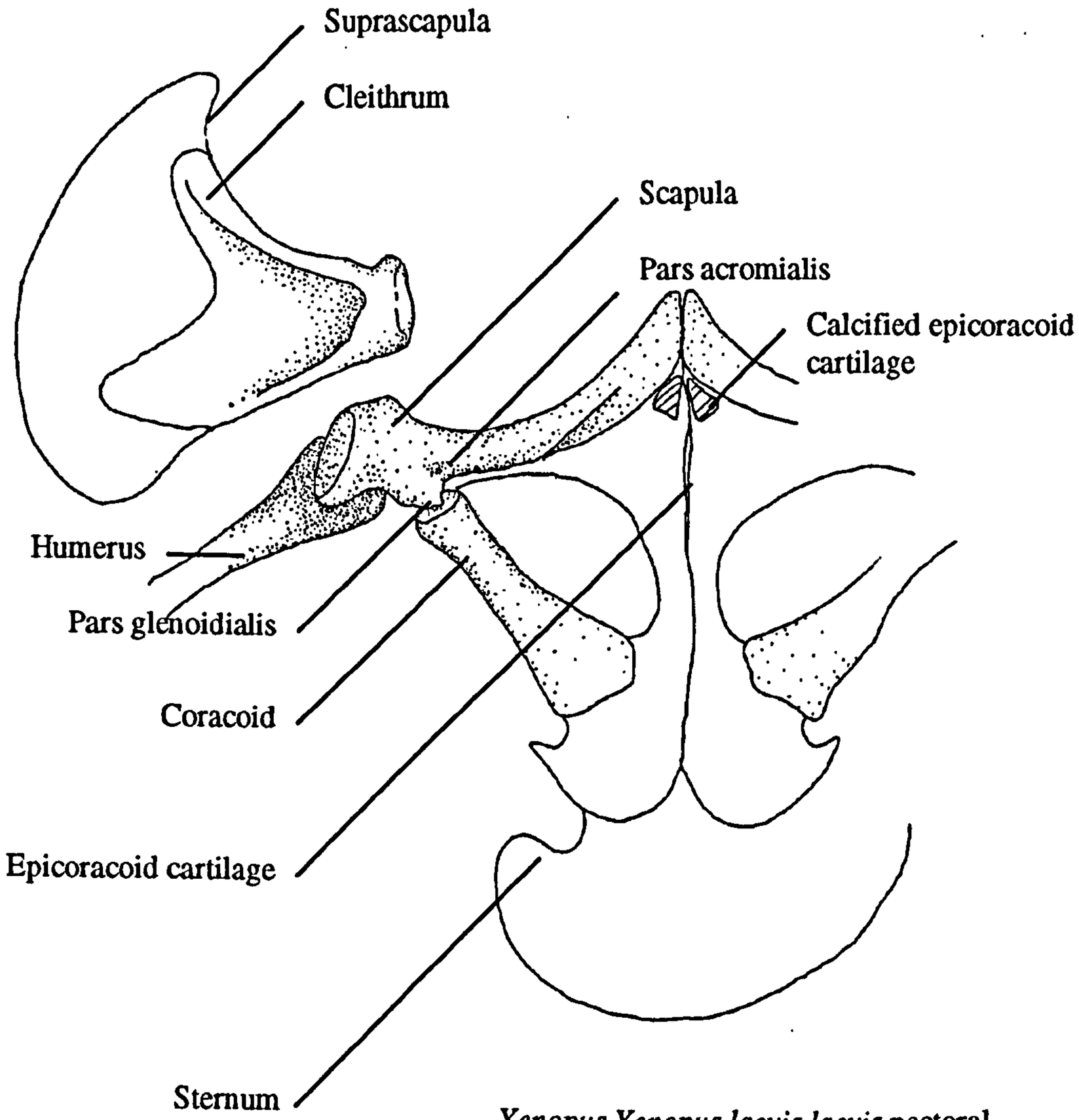
Four elements contribute to the pectoral girdle: cleithrum, coracoid, clavicle and scapula (Illustration 2-7). The latter two are indistinguishably fused in all *Xenopus*. The scapula associates by means of an oblique, lateral articulation with the cleithrum, which in turn extends posteromedially, overlapping mid-dorsally in some species.

Coracoids are long, narrow hourglass-shaped elements, which bear straight medial margins. They are dorsoventrally flat and are as wide, or wider, medially as they are laterally. The elements are narrowest just medial to the glenoid end, widening medially. They associate laterally by means of a glenoid cartilaginous bridge with the pars glenoidialis of the scapula and medially with epicoracoid cartilage. The angle formed between the main axis of the element and the zonal plane is inter-specifically variable, ranging from 35° to 60°.

Clavicles arch anteromedially, are slightly narrower laterally than are coracoids and bear a dorsal ridge that extends medially along the medial-most quarter of the element for accommodation of epicoracoid cartilages. Parallel, medial margins are separated by a very narrow gap. In some species, clavicle margins are parallel along their entire length, whilst in others, the element widens towards its medial margin, increasing in width by up to four-times medially. In such cases, the anterior profile is highly convex.

The scapula is roughly trapezoid-shaped with a convex anterior margin, concave posterior margin and an elongated posterolateral corner. It measures between one-third and a quarter the length of clavicles and bears a prominent, laterally directed process that articulates with the humeral condyle (Illustration 2-8ii). Partes acromialis and glenoidialis of the scapula are medially directed and may either be fused to one another or separated by a deep cleft. In the former case, there may or may not be a scar marking the site of synostosis between the two.

Associating dorsolaterally with the scapula are the fused, bifurcate cleithrum and suprascapula. The posterior blade of the former is considerably wider and slightly longer than the anterior, whose anterior margin is concave. This compound element has a well-developed groove extending from the inner scapular edge along approximately half of both the anterior and posterior margins, marking the insertion position of suprascapular cartilage. Calcification of pectoral cartilage occurs to varying degrees and is limited to the sternum, epicoracoid cartilages (particularly between coracoids and clavicles) and the suprascapula.



Xenopus Xenopus laevis laevis pectoral girdle

Illustration 2-7

Forelimb

The humerus, or proximal propodial element, articulates proximally with the lateral face of the scapula and distally with the compound, epipodial radio-ulna (Illustration 2-8, Illustration 2-7 and Illustration 2-9). Proximally, the lateral humeral epicondyle bears a deep crater into which a process on the lateral face of the scapula inserts during forward movement of the limb. This process has not previously described for this genus. Medial and lateral cristae are highly proliferated and are separated by a deep furrow that extends along the proximal third of the element. Sulci extend medially from both medial and lateral aspects of the distal condyle along approximately half the length of the element. The distal humeral condyle is accommodated by the olecranon, a modification of the radio-ulna that curves around its proximal condyle. The radio-ulna is twisted 90° along its main axis and bears sulci that extend from the straight, distal end of the element along approximately half its length. Lateral to the olecranon, and embedded in ligaments connecting humerus to radio-ulna is a small sesamoid element that provides structural and mechanical support for the joint.

Carpal elements (Illustration 2-9) comprise eight proximal cartilaginous elements, four digits and a single pre-axial structure, the prepollex. The ulnare lies proximal and post-axial to the ulna, and adjacent to the radiale. Element Y and the prepollex develop along the pre-axial axis. Remaining carpal elements, in post- to pre-axial sequence, are carpal elements four, three, two and one, which lie adjacent to the bases of metacarpals 1-4 respectively. The phalangeal formula is 2-2-3-3. A single sesamoid lies adjacent to the distal end of the radius along the pre-axial axis.

Posterior appendicular skeleton

Pelvic girdle

The pelvic girdle forms a U-shaped (V-shaped in *X. vestitus*) compound structure that varies in relative breadth and is composed of ischium and paired iliac and pelvic elements (Illustration 2-10). Paired iliac processes extend anteriorly or anterolaterally and bear dorsolaterally-projecting crests that extend distally along approximately three-quarters of their length, being widest at mid-length. Crests create a medial groove along the shaft that accommodates lateral margins of sacral diapophyses. Ilia are convex and associate proximally to form an inter-iliac symphysis. Dorsal prominences adjacent to this symphysis provide an attachment site for adductor muscles, *m. iliofibularis* and *m. iliofemoralis* (*sensu* Trueb, 1996). Laterally ilia form paired, dorso-ventrally compressed, oval sockets to accommodate the femur acetabulum in a ball-and-socket arrangement. Paired ischia form a laterally compressed ridge that extends along the dorsal face of the element. Paired pubic elements occur ventrally and provide a ventral site of attachment for the epipubis. Complete fusion between elements of the pelvic girdle occurs in some specimens, despite calcification in pubic elements being retarded.

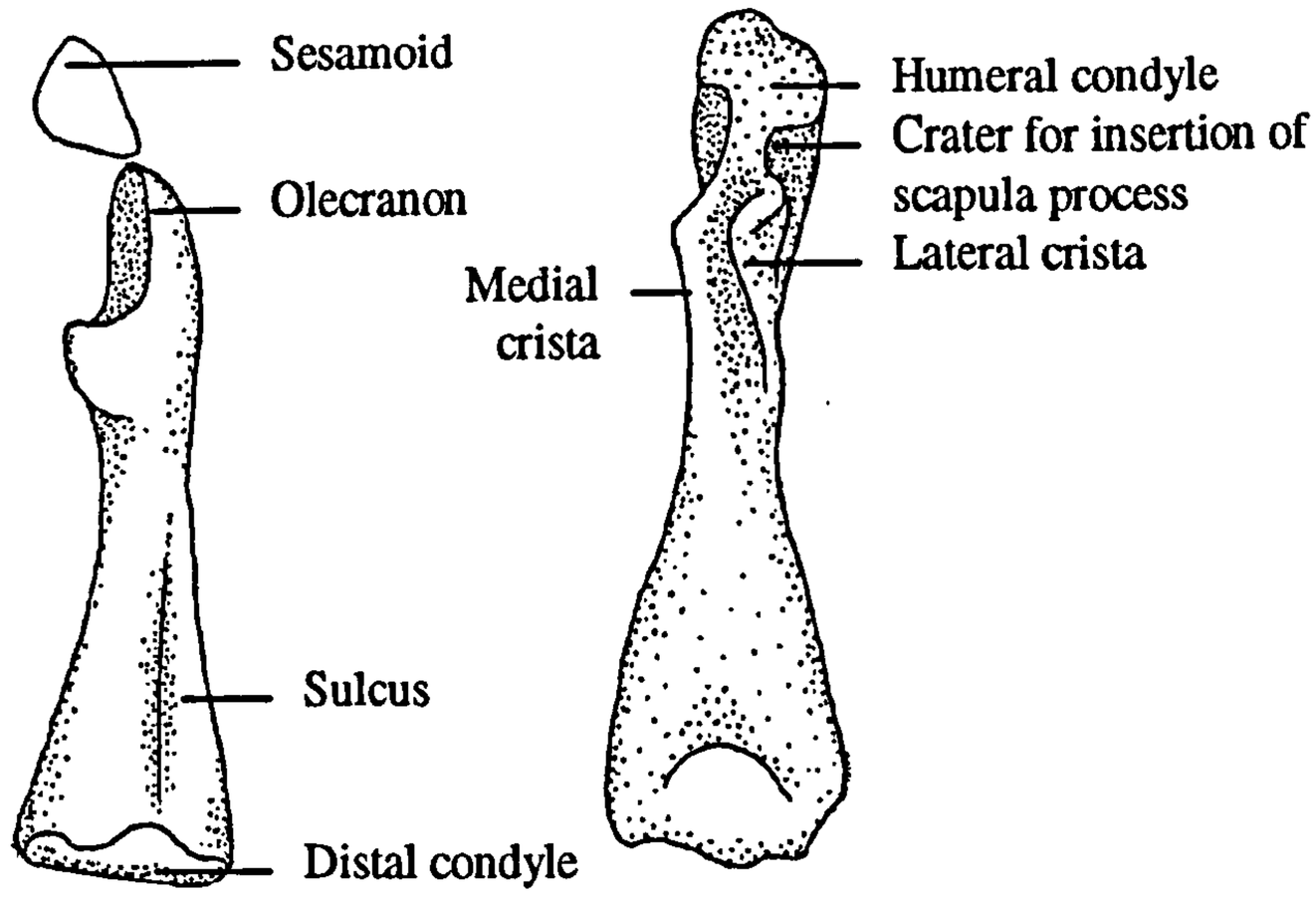
Hindlimb

The femur articulates distally with the compound tibia-fibula, or shank, which articulates in turn with the tibiale and fibulare, fused at both proximal and distal margins, and associating distally with tarsal elements (Illustration 2-11, Illustration 2-12).

The femur is a simple, sigmoid-shaped element that bears a modestly proportioned trochanter (*sensu* Duellman and Trueb, 1986) posteromedially on the proximal end of the shaft. Likewise, the compound tibia-fibula is simple and usually slightly longer than the femur. It lacks any distinguishing crests but has sulci extending from both ends. Whilst metatarsals IV and V articulate post-axially with the tibiale-fibulare indirectly via distal tarsal elements, metatarsals III and II do so via compound distal tarsal 3 and 2, and metatarsals II and I via distal tarsal 1, pre-axially. Element Y is ontogenetically related to the tibiale and is positioned dorsal to paired prehallical elements, which demonstrate inter-specific variability in relative lengths. The phalangeal formula is 2-2-3-4-3. The sequence of phalanx length, in ascending order, is 4-3-5-2-1.

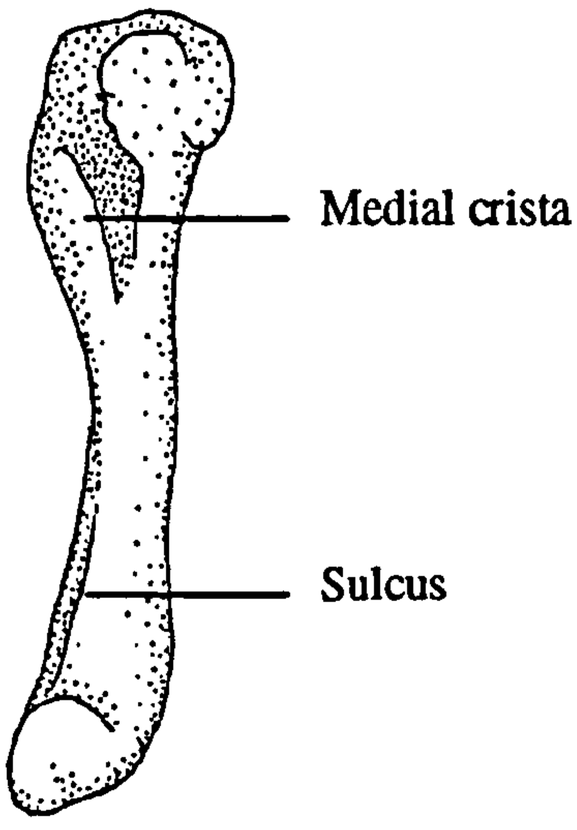
Sexual Dimorphism

In addition to features already noted for the larynx, the tympanic annulus is more dorsally oriented and larger in males than in females.



i. Dorsal view radioulna

ii. Anterolateral view humerus

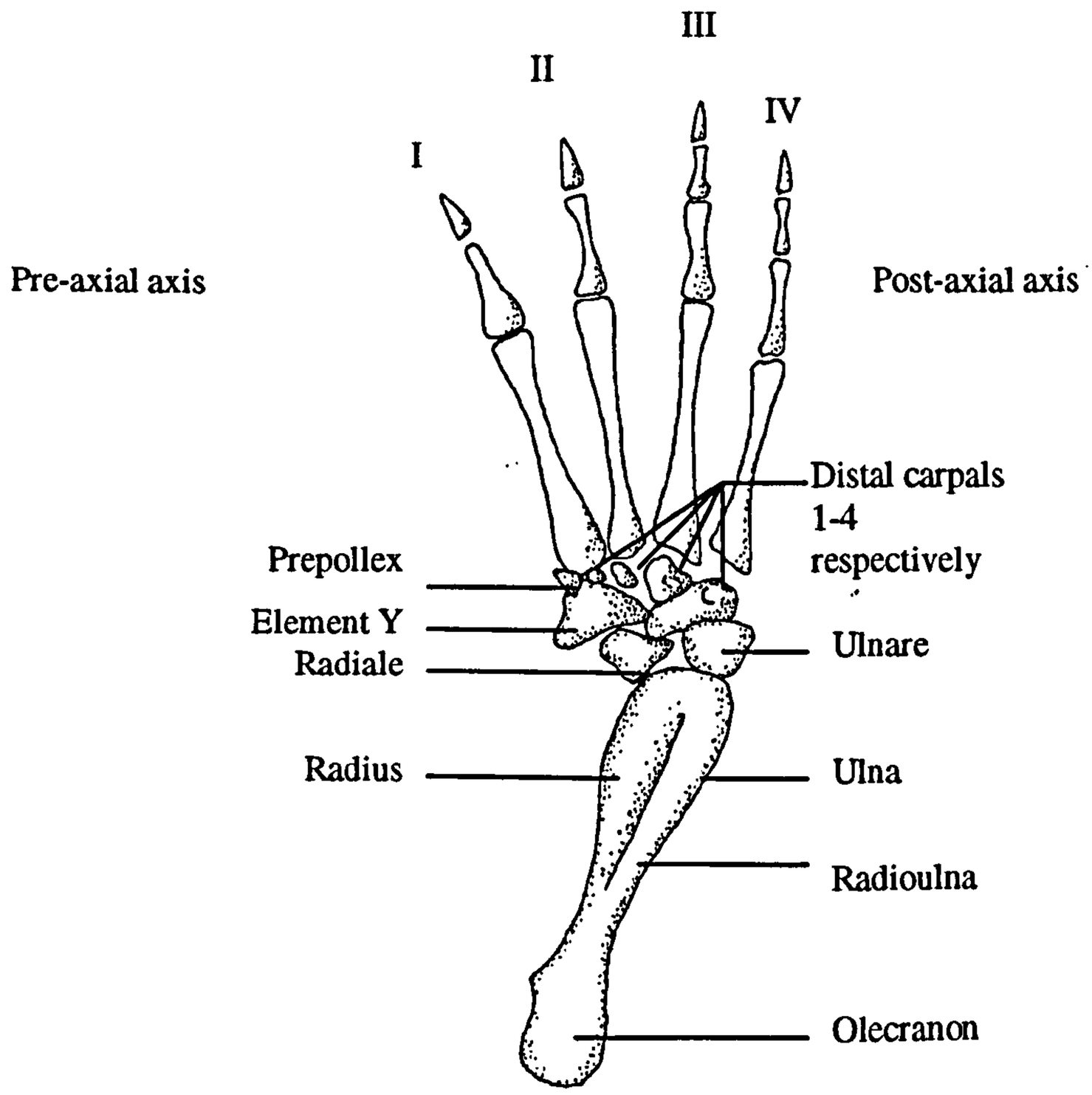


iii. Posteromedial view humerus

Xenopus laevis laevis

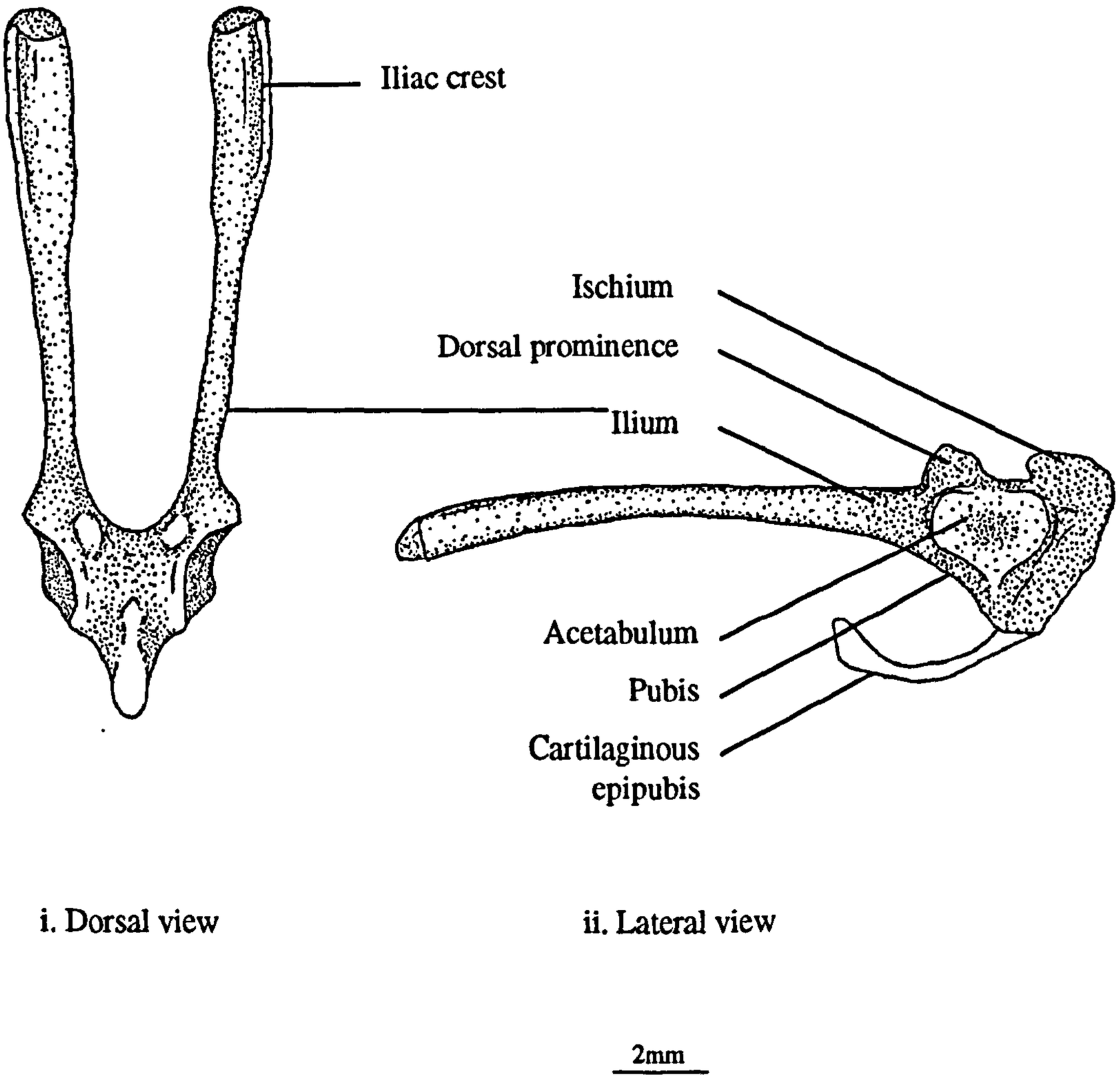
2mm

Illustration 2-8



Radioulna and carpal elements *Xenopus laevis laevis*

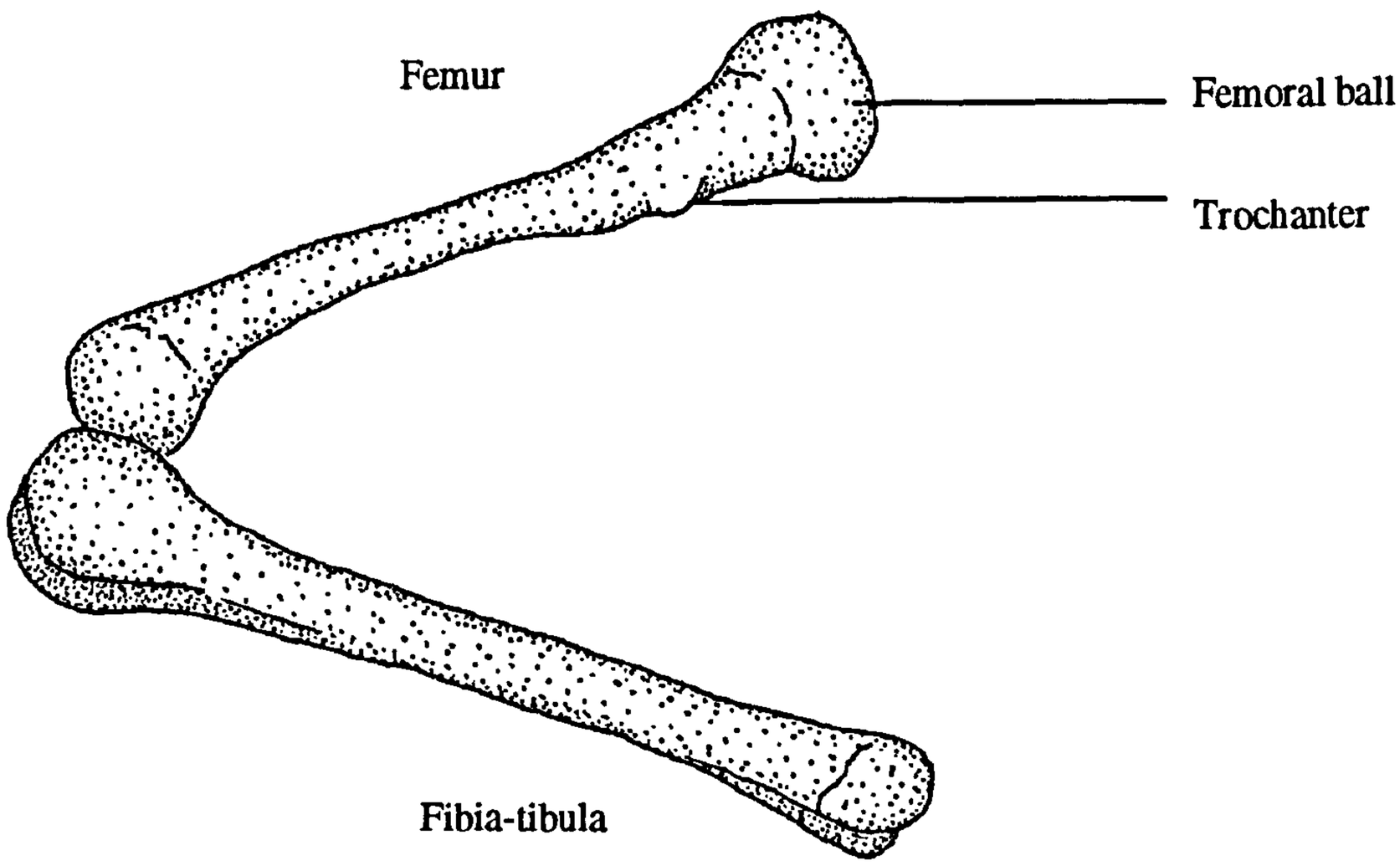
Illustration 2-9



Xenopus laevis laevis

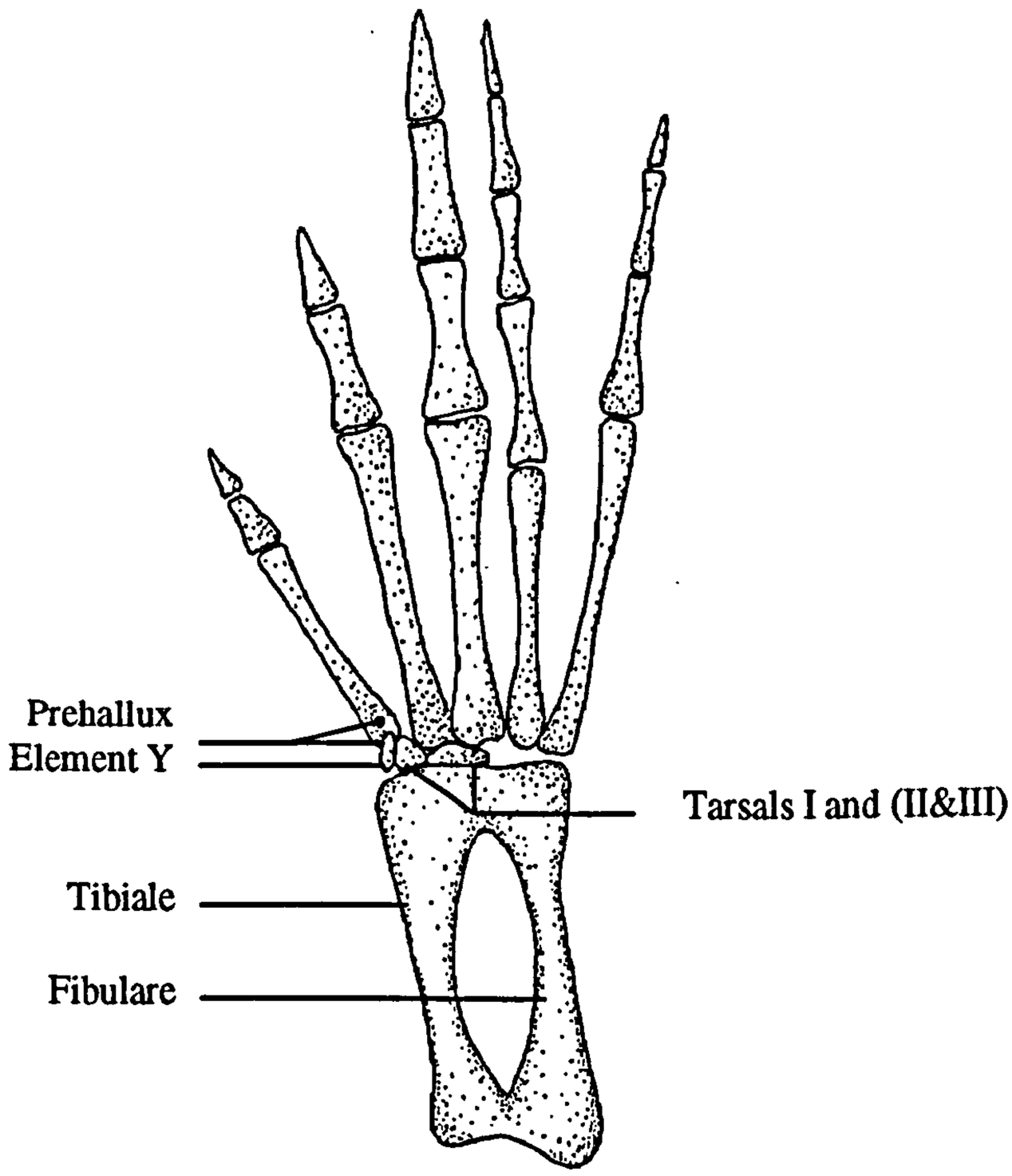
Pelvic girdle

Illustration 2-10



Long bones of the hindlimb

Illustration 2-11



Tarsus and metatarsals *Xenopus largeni*

2mm

Illustration 2-12

DISCUSSION

Many characters covered in this account have either not previously been described for *Xenopus*, have been erroneously described in the literature or have been found to vary more than has been documented in the literature. Those considered to be of taxonomic importance on the basis of information available from the literature are commented on below, with reference to the state reported for other extant Pipidae. Although the states of many more characters differ from literature accounts, it has been assumed that these are of little taxonomic significance since no documentation was found in the literature regarding their variability in other pipids.

Cranium

Much confusion surrounds the state of the vomer in *Xenopus*. Though Noble identified the element in *Xenopus* in 1931, it was later mistaken for the cultriform process by Paterson (1939), and said to be 'possibly present' but 'probably not' by Jurgens (1971). The vomer was subsequently reported as being azygous in *Xenopus* (see Reumer, 1985; Trueb, 1993; Cannatella and Trueb, 1988a; Cannatella and De Sa, 1993). Paired vomers however, have since been reported as occurring in *X. largeni* (see Baez and Trueb, 1997) and occasionally in *X. laevis* (see Smirnov, 1994). Though edentate in all other taxa, vomerine teeth have been reported for *X. muelleri* (see Estes, 1977; Henrici and Baez, 2001). The current study found vomers to be highly variable in shape, number of elements and dental status. Whilst generally, the vomer is azygous in all taxa, paired exceptions were found for all except *X. borealis*, being more prevalent in some taxa than in others. Occasionally in some taxa, the vomer was divided into three or four fragments. *Xenopus largeni* exclusively demonstrated the paired state, and whilst *X. muelleri* was the only taxon with a dentate vomer, this state was not universal within the taxon, and was more widespread among western samples. Absent in *Silurana*, *Hymenochirus*, and *Pipa*, the vomer is paired in other primitive Anura (Cannatella and Trueb, 1988; Baez and Trueb, 1997).

Orbitonasal foramina were reported as not being enclosed by bone in *Xenopus* by Trueb and Hanken (1992), and by Cannatella and Trueb (1988a). The current study has found that they are universally enclosed in *X. largeni*, and occasionally enclosed in *X. fraseri* and *X. wittei*. Illustrations by Cannatella and Trueb (1988a) showed them to be enclosed by bone in *Silurana* and *Hymenochirus*. Information pertaining to their state in other pipids is currently unavailable in the literature.

Fused nasals are a synapomorphy of *Silurana* (see Reumer, 1995; Cannatella and Trueb, 1988a; Baez and Pugener, 1998), and are believed to represent a primitive state (Baez and Trueb, 1997). Although fused nasals have not been observed in any specimen examined during the present

study, nasals in *X. longipes* were said to be paired (Loumont and Kobel, 1991), and have also been reported as being paired in some juvenile *X. laevis* (see Reumer, 1985).

Divided otic plates are a feature of *Xenopus* and *Silurana* whilst they are undivided in *Pipa* and *Hymenochirus* (see Baez and Trueb, 1997). The only exception found in the present study was *X. largeni*, in which otic plates exclusively demonstrated an un-divided state.

Vertebral Column

Atlas and axis were said to be 'paired or separate' in *Xenopus* by Kluge and Farris (1969) and paired by Estes (1973), Cannatella and Trueb (1988a,b), Cannatella and De Sa (1993), Trueb (1996) and Henrici and Baez (2001). Though generally paired in *Xenopus*, atlas and axis are universally fused in *X. largeni* (pers. obs.) and are fused occasionally in *X. amieti* and other [unspecified] taxa (Baez and Pugener, 1998; pers. obs.), though personal observations have shown that these aberrant fusions are usually accompanied by other vertebral deformities. Primitively, atlas and axis are paired (Lynch, 1973), but in *Silurana, Hymenochirus* and *Pipa* they are fused (Cannatella and Trueb, 1988a,b; Cannatella and De Sa, 1993; Trueb, 1996).

Straight transverse processes were reported as characterizing *Silurana* (see Cannatela and Trueb, 1988a,b; Cannatella and De Sa, 1993) and though curved in most examined taxa they were also moderately straight in *X. wittei* and *X. fraseri* and were reported as being straight for *X. longipes* Loumont and Kobel, 1991.

Cannatella and Trueb (1988a,b), Trueb and Cannatella (1986) and Baez and Trueb (1997) found *Pipa, Hymenochirus* and *Pseudhymenochirus* to differ from all other pipids in having paired neural spines. The current study has also found paired spines on all vertebrae of *X. muelleri* (east) and on the atlas of some *X. laevis* and *X. borealis* specimens. Neural spines were also reported to be under-developed by Trueb (1996). Dorsal ornamentation of neural spines however, has been found in the present study to be extensive in larger specimens of *X. laevis* and *X. muelleri.*

Pectoral girdle

Trueb (1996) stated that medial expansion of clavicles is a synapomorphy of *Xenopus*. Baez and Pugener (1998) extended this to include *Silurana*. The expanded state does not occur however, in *X. borealis* or *X. muelleri* (west) and occurs only to a modest degree in *X. fraseri* and *X. wittei.*

Other characters for which there is no taxonomic information

The scapula was said to be uncleft by Procter (1921) and Estes and Reig (1973) and cleft by Cannatella and Trueb (1988a,b), but has been found to vary intra-specifically in almost all taxa examined for the present study. Contrary to reports by Estes and Reig (1973), no mentomeckelian ossifications were found in any specimen examined during the present study. Finally, pineal foramina were present in all specimens, contrary to reports by Trueb and Cannatella (1986) that they were absent in some of the taxa that have been examined during the present study.

CONCLUSION

This study presents a previously undocumented degree of inter- and intra-specific variation in the osteology of the genus *Xenopus*. Despite the study being based on an incomplete representation of species within the genus, results indicate that the distinction between sub-genera *Silurana* and *Xenopus,* which though supported by non-osteological studies (Mann, Risley, Eckhardt and Kasinsky, 1982; Burki and Fischberg, 1985; Tymowska, 1977; Bisbee, Baker, Wilson, Hadji and Fischberg, 1977; Carr, Brothers and Wilson, 1987; Kobel *et al.*, 1981) is perhaps not as robust as had been suggested by previous osteology-based phylogenetic studies. Evidence is presented of forms that are intermediate between *Silurana* and *Xenopus*, one of which, *X. largeni*, will form the basis of chapter 8. Furthermore, results provide the information required for a re-assessment of the position of a number of *Xenopus* fossils whose phylogenetic position has recently been contested and also for the determination of the former extent of the distribution range of the genus.

BIBLIOGRAPHY

Ahl, E. (1926). "*Xenopus stromeri* Ahl, n. sp." In die Diamantenwuste Sudwest-Africas 2: 141-142. (Ed. Kaiser, E.) Verlag von Dietrich Reimer (Ernst Vohnsen), Berlin.

Bàez, A.M. (1981). Redescription and relationships of *Saltenia ibanezi*, a late Cretaceous pipid frog from northwestern Argentina. *Ameghiniana* **3-4**:127-154.

Bàez, A.M. 1996. "The fossil record of the Pipidae." Pp. 329-47 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Bàez, A.M. and Pugener, L.A. (1998). A new Palaeogene pipid frog from northwestern Patagonia. *Journal of Vertebrate Palaentology* **18(3)**:511-524.

Bàez, A.M. and Rage, J.C. (1998). Pipid frogs from the upper Cretaceous of In Beceten, Niger. *Palaentology* **41 (4)**:669-691.

Bàez, A.M. and Trueb, L. (1997). Redescription of the Paleogene *Shelania pascuali* from Patagonia and its bearing on the relationships of fossil and recent pipoid frogs. *Scientific papers, Natural History Museum, University of Kansas* **4**:1-41.

Bàez, A.M., Trueb, L. and Calvo, J.O. (2000). The earliest known pipid frog from South America: a new genus from the middle Cretaceous of Argentina. *Journal of Vertebrate paleontology* **20(3)**:490-500.

Bernasconi, A.F. (1951). Über den ossifikationsmodus bei *Xenopus laevis* Daud. *Denkschriften Der Schweizerischen Naturforschenden Gesellschaft* **79**:1-251.

Bisbee, Ch.A., Baker, M.A., Wilson, A.C., Hadji-Azimi, I. and Fischberg, M. (1977). Albumin phylogeny for clawed frogs (*Xenopus*). *Science* **195**:785-787.

Burki and Fischberg (1985). Evolution of globin expression in the genus *Xenopus* (Anura, Pipidae). *Molecular Biology and Evolution* **2(3)**:270-277.

Bustos, R.P., Caprini, M.F., Kraglievitch, J.L. and Del Corro, G. (1960). Noticia preliminar acerca del yacimiento de anuros extinguidos de puente morales. *Seperata de la revista de la facultad de ciencias naturales de Salta, Argentina.* **Tomo 1, 2**:5-25.

Cannatella, D.C. 1985. A phylogeny of primitive frogs. Preliminary manuscript for thesis chapter. Pers. comm.

Cannatella, D.C. and De Sá, R.O. (1993). *Xenopus laevis* as a model organism. *Systematic Biology* **42**:476-505.

Cannatella, D.C. and Trueb, L. (1988a). Evolution of pipoid frogs: inter-generic relationships of the aquatic frog family Pipidae (Anura). *Zoological Journal of the Linnean Society* **94**:1-38.

Cannatella, D.C. and Trueb, L. (1988b). Evolution of pipoid frogs: Morphology and phylogenetic relationships of *Pseudhymenochirus*. *Journal of Herpetology* **22** (4):439-456.

Carr, S.M., Brothers, A.Y. and Wilson, A.C. (1987). Evolutionary inference from restriction maps of mitochondrial DNA from nine taxa of *Xenopus* frogs. *Evolution* **41**:176-188.

Cope, E.D. (1864). On the limits and relations of the Raniformes. *Proceedings of the Academy of Natural sciences, Philadelphia* **16**:181-184

Duellman, W.E. and Trueb, L. 1986. Biology of Amphibians 1st ed. New York, St. Louis, San Francisco: McGraw-Hill Book company.

Estes, R. (1975a). Fossil *Xenopus* from the Palaeocene of South America and the zoogeography of pipid frogs. *Herpetologica* **31**:263-278.

Estes, R. (1975b). *Xenopus* from the Palaeocene of Brazil and its zoogeographic importance. *Nature* **254**:48-50.

Estes, R. (1975c). Relationships of the South African fossil frog *Eoxenopoides reuningi* (Anura, Pipidae). *Annals of the South African Museum* **73**:49-80.

Estes, R. (1977). Relationships of the South African fossil frog *Eoxenopoides reuningi* (Anura, Pipidae). *Annals of the South African Museum* **73**:49-80.

Estes, R. and Reig, O.A. (1973). "The early fossil record of frogs. A review of the evidence." Pp. 11-63 in Evolutionary Biology of the Anurans: Contemporary Research on Major Problems, J. L. Vial, ed. Columbia: University of Missouri Press.

Griffiths, I. (1963). The phylogeny of the Salientia. *Biological Reviews* **38**:241-292.

Henrici, A.C. (1998). A new pipoid anuran from the late Jurassic Morrison formation at Dinosaur National Monument, Utah. *Journal of Vertebrate Palaentology* **18 (2)**:321-332.

Henrici, A.C., and Baez, A.M. (2001). First occurrence of *Xenopus* (Anura:Pipidae) on the Arabian peninsula: A new species from the upper Oligocene of Yemen. *Journal of Palaeontology* **75(4)**:870-882.

Jurgens, J. (1971). The morphology of the nasal region of Amphibia and its bearing on the phylogeny of the group. *Annals of the University of Stellenbosch* **46, Serial A, 2**:1-146.

Kluge, A.G. and Farris, J.S. (1969). Quantitative phyletics and the evolution of anurans. *Systematic Zool.* **18(1)**:1-32.

Kobel, H.R., Du Pasquier, L. and Tinsley, R.C. (1981). Natural hybridization and gene introgression between *Xenopus gilli* and *Xenopus laevis laevis* (Anura: Pipidae). *Journal of Zoology, London* **194**:317-322.

Loumont, C. and Kobel, H.R. (1991). *Xenopus longipes* sp. nov., a new polyploid pipid from western Cameroon. *Revue Suisse Zoologie* **98 (4)**:731-738.

Lynch, J.D. (1969). Evolutionary relationships and osteology of the frog family Leptodactylidae. Ph.D. Dissertation, University of Kansas.

Lynch, J.D. (1973). "The transition from archaic to advanced frogs". Pp.133-185 in Evolutionary Biology of the Anurans: Contemporary Research on Major Problems, J. L. Vial, ed. Columbia: University of Missouri Press.

Mann, W., Risley, M.S., Eckhardt, R.A. and Kasinsky, H.E. (1982). Characterization of sperm/spermatid basic chromosomal proteins in the genus *Xenopus* (Anura: Pipidae). *Journal of Experimental Zoology* **200**:173-186.

Nevo, A. (1956). Fossil frogs from a lower Cretaceous bed in southern Israel (Central Negev). *Nature* **178**:1191-1192.

Noble, G. (1922). The phylogeny of the Salientia. I- the osteology and thigh musculature: their bearing on classification and phylogeny. *Bulletin of the American Museum of Natural History* **46**:1-87.

Noble, G. 1931. The biology of the Amphibia. McGraw-Hill Book Co., New York.

Nicholls, G.C. (1916). The structure of the vertebral column in Anura, Phaneroglossa and its importance as a basis of classification. *Proceedings of the Linnean Society, London (Zoology)* **128**:80-92

Orton, G.L. (1953). The systematics of vertebrate larvae. *Systematic Zoology* **2(2)**:63-75

Orton, G.L. (1957). The bearing of larval evolution on some problems in frog classification. *Systematic Zoology* **6(2)**:79-86.

Paterson, N.F. (1939). The head of *Xenopus laevis*. *Quarterly Journal of Microscopical Science* **81**:161-234.

Paterson, N.F. (1945). The skull of *Hymenochirus curtipes*. *Proceedings of the Zoological Society of London* **115**:327-354.

Paterson, N.F. (1955). The skull of the toad, *Hemipipa carvalhoi* Mir. Rib. with remarks on other Pipidae. *Proceedings of the Zoological Society of London* **125**:223-252.

Procter, J.B. (1921). On the variation in the scapula in the batrachian groups Aglossa and Arcifera. *Proceedings of the Zoological Society, London* **1-2**:197-214.

Reig, O.A. (1958). Proposiciones para une nueva macroistematica de los anuros. *Nota preliminare, Physis* **21**:109-118

Reig, O.A. (1959). Primeros datos descriptivos sobre los anuros del Eocretaceo de la provincia de Salta (Rep. Argentina). *Ameghiniana* **4**:3-8.

Reumer, W.F. (1985). Some aspects of the cranial osteology and phylogeny of *Xenopus* (Anura: Pipidae). *Revue Suisse Zoologie* **92 (4)**:969-980.

Sassoon, D. and Kelley, D. (1986). The sexually dimorphic larynx of *Xenopus laevis*: development and androgen regulation. *American Journal of Anatomy* **177**:457-472.

Sedra, S.N. and Michael, M.I. (1957). The development of the skull, visceral arches, larynx, and visceral muscles of the South African clawed toad, *Xenopus laevis* (Daudin) during the process of metamorphosis (from stage 55-66). *Vehrhandelingen Akademie Wetenschappen Amsterdam* **41(4)**:1-80.

Smirnov, S.V. (1994). Postmaturation skull development in *Xenopus laevis* (Anura, Pipidae): late-appearing bones and their bearing on the pipid ancestral morphology. *Russian Journal of Herpetology* **1, 1**:21-29.

Tihen, J.A. (1965). Evolutionary trends in frogs. *American Zoologist* **5**:309-318

Trueb, L. 1973. "Bones, Frogs and Evolution."Pp. 65-132 in Evolutionary Biology of the Anurans: Contemporary research on Major Problems, J. L. Vial, ed. Columbia: University Missouri Press.

Trueb, L. 1993. "Patterns of Cranial Diversity Among the Lissamphibia." Pp. 255-343 in The Skull. Vol. 2, edited by Hanken, J. and Hall, B.K. Chicago: The University of Chicago.

Trueb, L. (1996). "Historical constraints and morphological novelties in the evolution of the skeletal system of pipid frogs (Anura: Pipidae)." Pp. 349-376 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Trueb, L. (1999). The early Cretaceous pipoid anuran, *Thoraciliacus*: redescription, revaluation, and taxonomic status. *Herpetologica* **55(2)**:139-157.

Trueb, L. and Hanken, J. (1992). Skeletal development in *Xenopus laevis* (Anura:Pipidae). *Journal of Morphology* **214**:1-41.

Trueb, L. and Cannatella, D.C. (1986). Systematics, morphology and phylogeny of the genus *Pipa* (Anura: Pipidae). *Herpetologica* **42**:412-449.

Trueb, L. and Massemin, D. (2001). The osteology and relationships of *Pipa aspera* (Amphibia: Anura: Pipidae), with notes on its natural history in French Guiana. *Amphibia-Reptilia* **22**:33-54.

Trueb, L., Pugener, L.A. and Maglia, A.M. (2000). Ontogeny of the bizarre: An osteological description of *Pipa pipa* (Anura: Pipidae), with an account of skeletal development in the species. *Journal of Morphology* **243**:75-104.

Tymowska, J. (1977). A comparative study of the karyotypes of eight *Xenopus* species and subspecies possessing 36-chromosome complement. *Cytogenetics and Cell Genetics* **18**:165-181.

Vergnaud-Grazzini, C. (1966). Les amphibiens du Miocène de Beni-Mellal. *Notes du Service geologique du Maroc* **27, 198**:43-69.

Wassersug, R.J. (1976). A procedure for differential staining of cartilage and bone in whole formalin-fixed vertebrates. *Stain Technology* **51 (2)**:131-133.

CHAPTER 3 : THE EFFECTS OF THREE NATURAL VARIABLES, SEX, AGE AND SIZE ON THE OSTEOLOGY OF *XENOPUS LAEVIS LAEVIS*

INTRODUCTION

For most ectothermic animals, growth stops at sexual maturity (Miaud and Guyatant, 1998; Miaud, Guyatant and Elmberg, 1999). Growth in amphibians however, specifically in females, is known to continue indefinitely (Deuchar, 1975; Duellman and Trueb, 1986; McCoid and Fritts, 1989). During preliminary investigative studies of inter-species osteological variation, I found there to be a high degree of intra-specific osteological variation within adult samples. This was exacerbated in the case of females, so much so in some instances as to preclude the identification of inter-specifically variable osteological characters. Many of these characters appeared to vary primarily as a function of size, indicating an allometric pattern of growth; the pattern of variation in other characters however, was less distinct and did not appear to relate specifically either to growth nor sex.

Several authors have implicity assumed that variability in a number of osteological characters in *Xenopus* taxa is a function of age (Estes and Reig, 1973; Reumer, 1985; Trueb and Hanken, 1992; Smirnov, 1994). This explanation has been based on inference alone however, and is founded on the widespread misconception that size is proportional to age (Darwin, 1871; Duellman and Trueb, 1986), a relationship that has been refuted in repeated studies on Anura (Hemelaar, 1985; Leclair and Castanet, 1987; Halliday and Verrell, 1988; Ryser, 1988; Francillon-Vieillot, Arntzen and Geraudia, 1990; Cherry, 1992; Castanet, Francillon-Vieillot, Meunier and Ricqles, 1993; Tinsley and Tocque, 1995). Whether this variability is a function of size, age, or any of a range of other possible variables has yet to be determined, since no published account exists which documents the causes behind intra-specific osteological variability in Anura. Gaining an understanding of the form and degree of intra-specific osteological variation is of integral importance for any study concerned with inter-specific comparison, not only between extant taxa, but also to evaluate the phylogenetic position of extinct forms in this respect, since osteology is the only means by which this can be achieved.

Only since the late 1970's, with the advent of reliable techniques for ageing Anura, has it become possible to evaluate the effects of age on osteology in a wild sample. Skeletochronology is considered by many authors to be among the most effective ways of determining age (Halliday and Verrell, 1988; Castanet and Smirina, 1990; Castanet *et al.*, 1993; Miaud, Andreone, Riberon, Michelis, Clima, Castanet, Francillon-Vieillot and Guyetant, 2001). The method employs growth marks laid down in bone at variable rates as a tool to obtain temporal data that enable the assessment of growth rate (Castanet *et al.*, 1993). The deposition of growth marks is primarily a function of extrinsic environmental factors acting locally on the population as a whole, and is of greatest value in

areas of high seasonality (Castanet and Baez, 1991). Growth marks or lines of arrested growth (LAG) represent periods of arrested growth and are best demonstrated in long, thin bones. They are separated by wide zones of periosteal, lamellar bone that is laid down during growth bursts. The zones separating rings are wide in immature animals, narrowing upon maturity. This difference in bandwidth represents the physical expression of a change in the allocation of energy resources from somatic growth to reproduction (Halliday and Verrell, 1988). Bands become narrower still in old age, a phenomenon known as raproachment (Hemelaar, 1985; Francillon-Vieillot *et al.*, 1990; Castanet *et al.*, 1993).

Skeletochronology has proved invaluable for the study of life history and population age structures, and holds great potential for studies of age-dependent changes in shape, size and structure in bone (Castanet *et al.*, 1993). By the very nature of this technique however, a range of problems can complicate interpretation of LAG. A common assumption has been that LAG are laid down annually, usually during periods corresponding to low food availability (Hemelaar, 1985; Leclair and Castanet, 1987; Halliday and Verrell, 1988; Castanet *et al.*, 1993). Since it is known that LAG are laid down during periods of torpor, the periodicity of production is unique to each model group and by definition depends on local climate and food availability (Halliday and Verrell, 1988; Castanet *et al.*, 1993). Few studies have validated the periodicity of the deposition of LAG in bone, of these both circannual and biannual patterns have been demonstrated for temperate species (Measey and Wilkinson, 1998). It is not known however, whether the method is valid for tropical species (Halliday and Verrell, 1988). In practice, lines may be imperfect, making their interpretation troublesome. Occasionally, double LAG are laid down within one growth season (Caetano, Castanet and Francillon, 1985). Without detailed ecological information, however it is not clear how such marks should be interpreted in different model groups. Tinsley and Tocque (1995) reported 30 days fasting/dormancy in a desert anuran as being necessary for the formation of a LAG. This period is likely to differ between anuran groups however, and there are no published accounts pertaining to *Xenopus*. Shadow lines represent a similar problem (Caetano *et al.*, 1985), extending around the entire circumference in some bone sections, whilst fading partially in others. Resorption of endosteal bone at the periphery of the medullary cavity, and subsequent loss of innermost rings is also a problem in skeletochronology (Leclair and Castanet, 1987; Hemelaar, 1988). Despite attempts by Hemelaar (1988) to overcome this problem using statistical methods, intra-specific variability in life history introduces a number of variables that affect the rate of LAG deposition, which cannot be accurately factored into such analyses.

Despite methodological advancements for age determination in Anura, very few skeletochronological studies have been conducted on *Xenopus* species. A feral population of *X. l. laevis* in south Wales has been the subject of a long-term population study (Measey, 1997; Measey and Tinsley, 1998) and although originally, ageing of specimens was by mark-recapture, more

recently, skeletochronology has been applied to the study of their life history and population dynamics. The only study specifically conducted to place osteological variation in *Xenopus* into an age context was by Smirnov (1994), who examined cranial osteology in a small sample of under-sized (see Kobel, Loumont and Tinsley, 1996), lab-reared *X. l. laevis*. Ages varied between 2 and 9 years. One specimen of each sex was used for age categories 2, 5 and 7 years, and a single female specimen for each of age categories 9 and 12 years. Smirnov described an aberrant osteological feature, the palatine bone (unrecorded in other studies for *Xenopus*) in one 9 year-old specimen, which was absent in all others and explained in terms of its late appearance and ultimate fusion with the vomer, despite there being no evidence of a suture on the vomer at the site of its supposed fusion. It was also concluded that the vomer, azygous in 2 and 5 year olds, medially notched in a 7 year old and paired in the 9 year-old, is paired in older specimens. Smirnov's explanation for his observations implies a sequential pattern of development for these and other elements in *X. l. laevis*. His sample sizes were insufficient however, to represent the extent of natural variation that exists within this taxon (pers. obs.), and are too small to draw conclusions about the directionality of trends with age. However, the study served to highlight a degree of intra-specific variation previously undocumented in the literature.

It became apparent early during my own investigations comparing osteology between *Xenopus* taxa that indeed, a high degree of intra-specific osteological variation exists for many *Xenopus* species. This complicated the task of distinguishing inter- from intra-specific variation, especially in the case of phenetically similar taxa. These findings made clear the need for a study that attempted to quantify this variation and to explain it within the context of known criteria, where possible. A number of natural variables affect all wild anuran populations, three of which, namely sex, age and size can be determined without the additional need for detailed ecological information pertaining to their site of origin.

AIM

A large, wild-caught sample was collected from a feral population of *X. l. laevis* living in California and presented an unusual opportunity to investigate the degree and form of intra-specific variation within this taxon. Osteology will be examined in this sample and the relationship between sex, age and size investigated. The differential effects of each of these variables on osteology will be assessed. The study will serve not only to determine the degree of natural variation in the taxon, but also to differentiate between the most and least variable osteological characters, thereby identifying the most dependable specific indicators for *X. l. laevis*.

MATERIALS AND METHODS

A large sample of feral *X. l. laevis* was trapped from Avocado Pond, Spring Valley (San Diego, California) by Richard Tinsley in 1989. This area experiences a Mediterranean climate, with a drought period typically extending from April through until October, and animals normally reproducing for a period of up to 3 months a year (McCoid and Fritts, 1989). All specimens were collected at the same time from the same site, minimizing the confounding effects of differing exposures to a locally variable climate. Some degree of intra-specific variability in the rate of deposition of LAG is inevitable however, owing to the effects of intra-specific competition, skill and chance in prey-capture, immigration from other sites *et cetera*. Animals were trapped using modified fyke-nets baited with liver and steak (see Measey, 1997 for site details).

Skeletochronology

Skeletochronology was used to determine relative ages within the sample, and to ensure the inclusion of specimens spanning a range of ages. Since it was not possible to validate the periodicity of the laying down of LAG in this population, age-units will be discussed in terms of LAG, rather than months/years, whilst not precluding the possibility that one LAG may actually represent one year's growth.

Bone sectioning

Animals had been fixed and stored in 10% formalin. Femurs were removed, the shaft diaphysis isolated and decalcified in 2% formal nitric decalcifying fluid (1:20 bone-to-fluid volume). Solutions, containing bone diaphyses, were placed on a rotating board for up to 7 days (replacing solution daily). Decalcification was tested by pipetting 15 drops of ammonium hydroxide into 5ml of decalcifying solution. If clear after 1 minute, 0.5 ml saturated ammonium oxalate was added and left for a further 30 minutes. A clear solution indicated that decalcification was complete. Femurs were then neutralized for 12 hours in 5% sodium-sulphate, and dehydrated through an ethanol series before immersion in Histolene for three hours, replacing with fresh solution every hour. Samples were then placed into a mixture of molten paraffin wax (BDH 36133; melting point 56°C) and Histolene (1:1) at 60°C, and placed in a vacuum of -760mm Hg. After an hour, specimens were transferred into vials containing pure molten paraffin wax and returned to the same pressure and temperature regime for a further hour. Femurs were then set into pure paraffin wax and quickly cooled by immersion in iced-water prior to trimming off excess wax and slicing into 11µm thick sections on a rotary-microtome. Wax ribbons were floated in water over a 25°C hotplate to allow expansion of sections prior to transfer onto glycerin albumen-smeared slides on a 50°C hotplate to secure adhesion. Sections were de-waxed by immersion in Histolene for 20 minutes (2x10 minutes) prior to serial ethanol hydration; 100% for 20minutes (2 changes), 90% and 70% (10 minutes) and distilled water (5 minutes). Sections

were stained in Ehrlich's Haematoxylin (15 minutes), rinsed in tap water, differentiated in Acid alcohol (5 seconds) (0.25%HCl in 70% ethanol), immersed in Scott's tap water (5 minutes) and 70% ethanol (3 minutes) prior to counter-staining in alcoholic Eosin (30 seconds). Sections were dehydrated through an ethanol series; 70%, 90%, 2x100% (1 minute) before immersion in Histolene pre-mount (2 minutes x3), mounted in DPX (BDH 36029 4H) with cover slips placed to protect all sections. Examination of sections was carried out using a Nikon Optiphot compound microscope, and photographs taken with a Nikon UFX-IIA photomicrographic attachment and a Nikon motorized dark box.

The shaft diaphysis is said to exhibit the clearest LAG (Castanet *et al.*, 1993). From this portion, sections displaying the most distinct growth marks and minimum resorption were chosen for examination. If each of two bands in a double LAG pair extended around the entire circumference of the bone, both were counted. If one band was partially faded, the double band was counted as one. Likewise, shadow bands sometimes occurred in bone sections; again, these were counted if the shadow extended around the entire circumference of the bone. Where the line of metamorphosis was not visible, indicating resorption of endosteal bone (Hemelaar, 1988), specimens were not included in the study. Only sections showing no evidence of resorption or asymmetrical resorption, where one side of the medullary cavity was left intact, were used.

Each sample was analyzed three times, non-consecutively, by 2 individual observers without prior knowledge of animal size or sex. Where results differed between observers, sections were re-assessed by both parties until agreement was reached.

Clearing and Staining

Methods follow those outlined in chapter 2.

Statistical Methods

A total of 74 characters was assessed (60 continuous variables measured, and 15 characters scored on the basis of qualitative variation) at a magnification of x6 using an ocular micrometer fitted to a stereo binocular microscope (Leica), under fibre-optic illumination. The angle between coracoid and zonal axis was determined from tracings obtained using a Camera Lucida, and tooth counts taken for premaxillary and maxillary elements. Characters are listed in Appendix 1 of this chapter.

A test for repeatability of measures was carried out by comparing variation among measurement repeats with variation among individuals, on a subset of six individuals. Each specimen was measured six times non-consecutively. One-way analysis of variance was conducted on each variable, with individual and repeat as factors. Variables for which among-repeat variation exceeded among-individual variation were not considered repeatable (Harper, 1994).

Regression was used to investigate bivariate relationships between size, age and sex. Two sample t-tests and one-way analysis of variance were carried out on each variable in turn, standardized for snout-vent length (except for tooth-counts and coracoid/zonal plane angle), to test for the effects on osteology of sexual dimorphism and age respectively. Data in the case of t-tests, and residuals in the case of analysis of variance, were tested for normality. Data were also tested for homogeneity of variance. Log_e transformation was carried out in the event of data deviating from normal.

For multivariate analyses, data were log_e transformed to render morphometric variation more linear, to eliminate differences of scale between variables (Thorpe, 1983; Klingenberg, Spence and Mirth, 2000) and to achieve normality (Zar, 1984). Analyses were carried out on a correlation matrix, used to standardize the magnitude of variables and their variances (Thorpe, 1983). Assumptions that data were multivariate normal for PCA (principal component analysis) and DFA (discriminant function analysis), and that matrices had equal variance/ covariance for DFA were ignored, since no statistical inference was drawn from results and non-conformity to these assumptions are not thought to invalidate results (Butcher, 1980; Dillon and Goldstein, 1985; Rayner, pers. com.). Pooled within-group character relationships were specified in quadratic DFA to prevent confusion between within- and between-group differences (Thorpe, 1976, 1983), and prior-probabilities were computed from group size.

Principal component analysis was carried out to assess overall variation in the sample. Size was represented by the first component whilst remaining components represented size-independent, shape variation. It was therefore possible to use the multivariate size-correction method of Thorpe (1983) in order to negate the effects of size differences within- and between samples for multivariate analysis. This was done by conducting stepwise DFA on all but the first principal component factors, with sex, age- and size-category as grouping variables, enabling the analysis to focus exclusively on shape variation between groups.

Coefficients from the first DFA function were ranked, and cross-correlations drawn with their associated PCA character coefficients to give a measure of the relative discriminatory importance of each variable. DFA was also used to determine whether specimens could be correctly-classified according to sex, age- or size-category from osteometric data alone.

Both univariate and multivariate analyses were conducted as both methods may yield differing results depending on the degree of correlation between variables (Willig, Owen and Colbert, 1986) and the differing effects of standardizing for snout-vent length in the case of the former and overall size in the latter. A two-sample t-test and one-way analysis of variance was conducted to test the significance of relationships between DFA1 scores and sex, age- and size- categories respectively.

Statistical analysis was carried out on Minitab v. 10 for Windows ™ (Ryan *et al.*, 1985) and SPSS (10) and MVSP 3.2 (Kovach Computing).

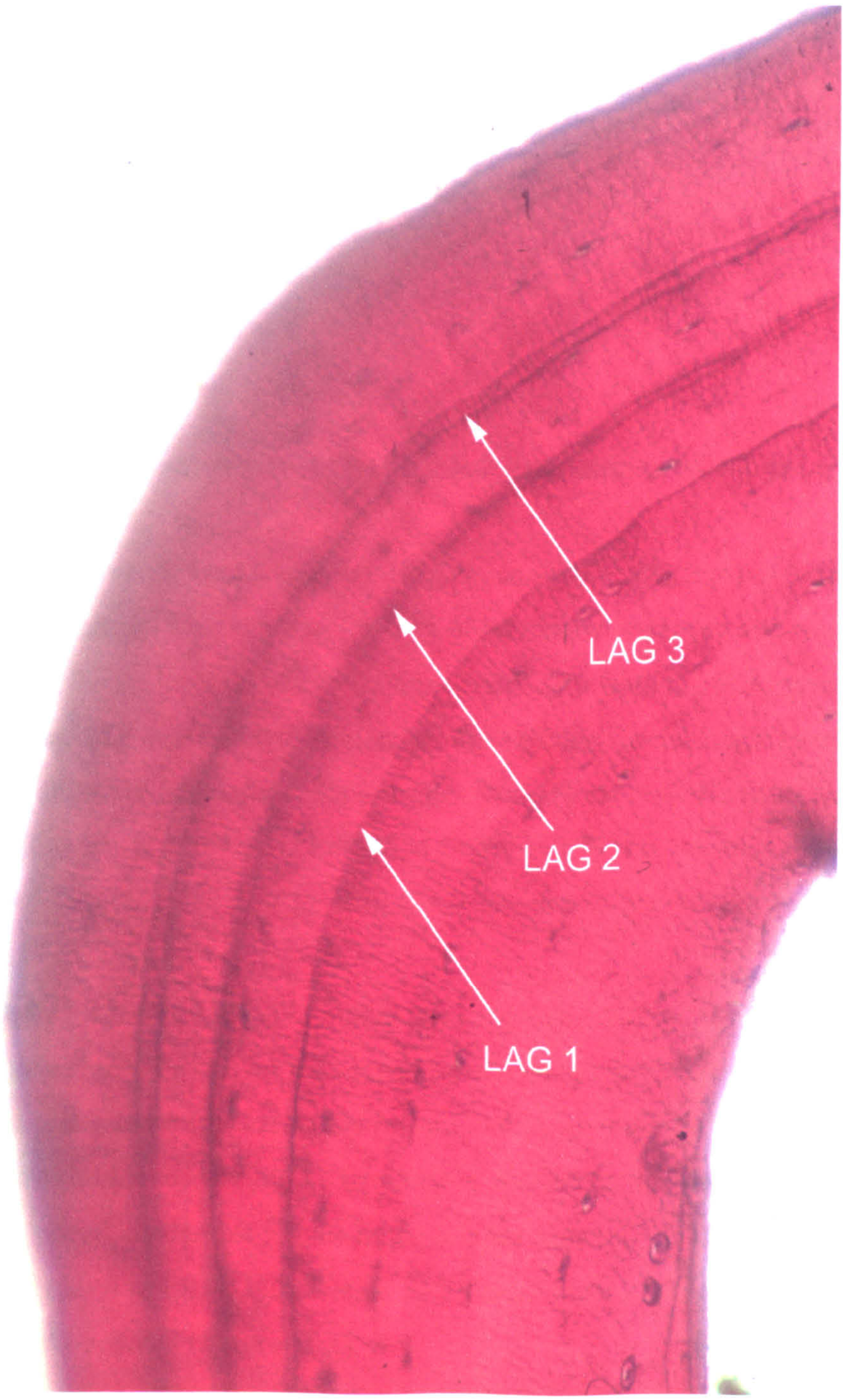
RESULTS

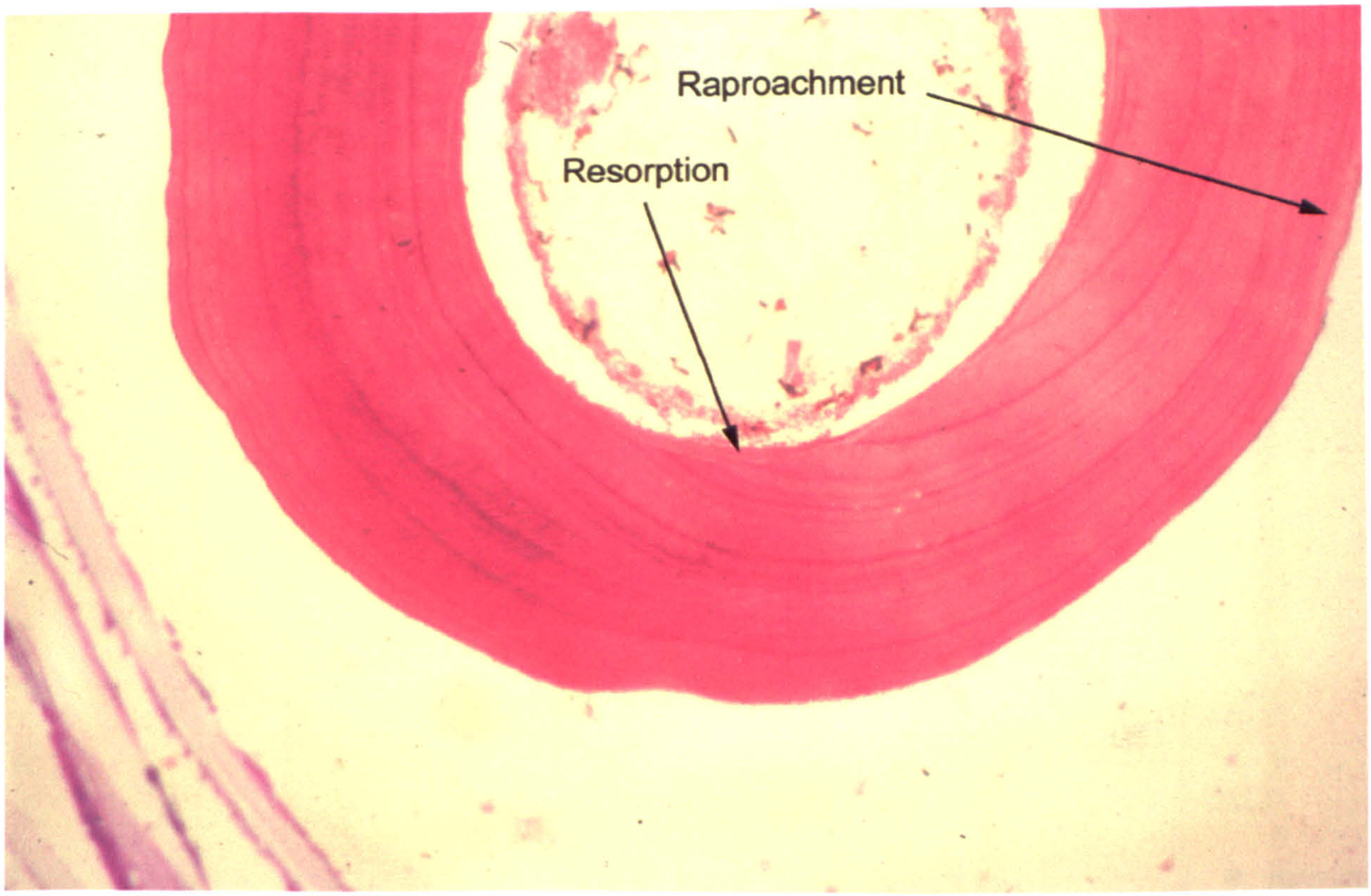
Age was determined for many more specimens than were used in the present study so as to select males and females to represent the maximum available age- and size-range. Thirty-four specimens were chosen for osteological examination and included 11 males (28-71mm; 0-7 LAG) and 23 females (31-81mm; 0-14LAG) (Figure3-3).

Figure 3-1

Photograph of bone section showing lines of arrested growth.

Scale: _____ 0.1mm.





Scale: _____ 0.1mm.

Figur3-2

Photograph of bone section showing resorption of endosteal bone and raproachment with old age.

All osteometric measurements were found to be highly, or very highly repeatable (*sensu* Harper, 1994). Data for femur length were missing for 44% of specimens owing to removal of both femurs on separate occasions for the purpose of age determination; data from the first ageing were not available for the current study. The distal prehallux element was missing from a single specimen, but mean lengths of remaining distal prehallux elements were not found to vary significantly between sexes or age groups ($t=0.77$, d.f.=21, $p=0.45$ and $F=0.36$, d.f.=29, $p=0.79$ respectively). Rather than replacing missing values with an estimate, data for both femur and pre-hallux lengths was excluded from subsequent analyses.

Snout-vent measures for both males and females were regressed on age. Both size and age ranges in the female sample exceeded those for males, and results from regression analysis showed the age-size relationship in males to be approximately linear ($F=85.1$, $p=0.000$) whilst in females, the relationship between these variables became highly irregular in specimens with more than 4 LAG ($F=32.4$, $p=0.05$ females). Indeed, the largest female in the sample had only 4LAG, and was considerably larger than the oldest at 14LAG, indicating an indeterminate and highly variable pattern of growth for females older than 4LAG (Figure- 3-3).

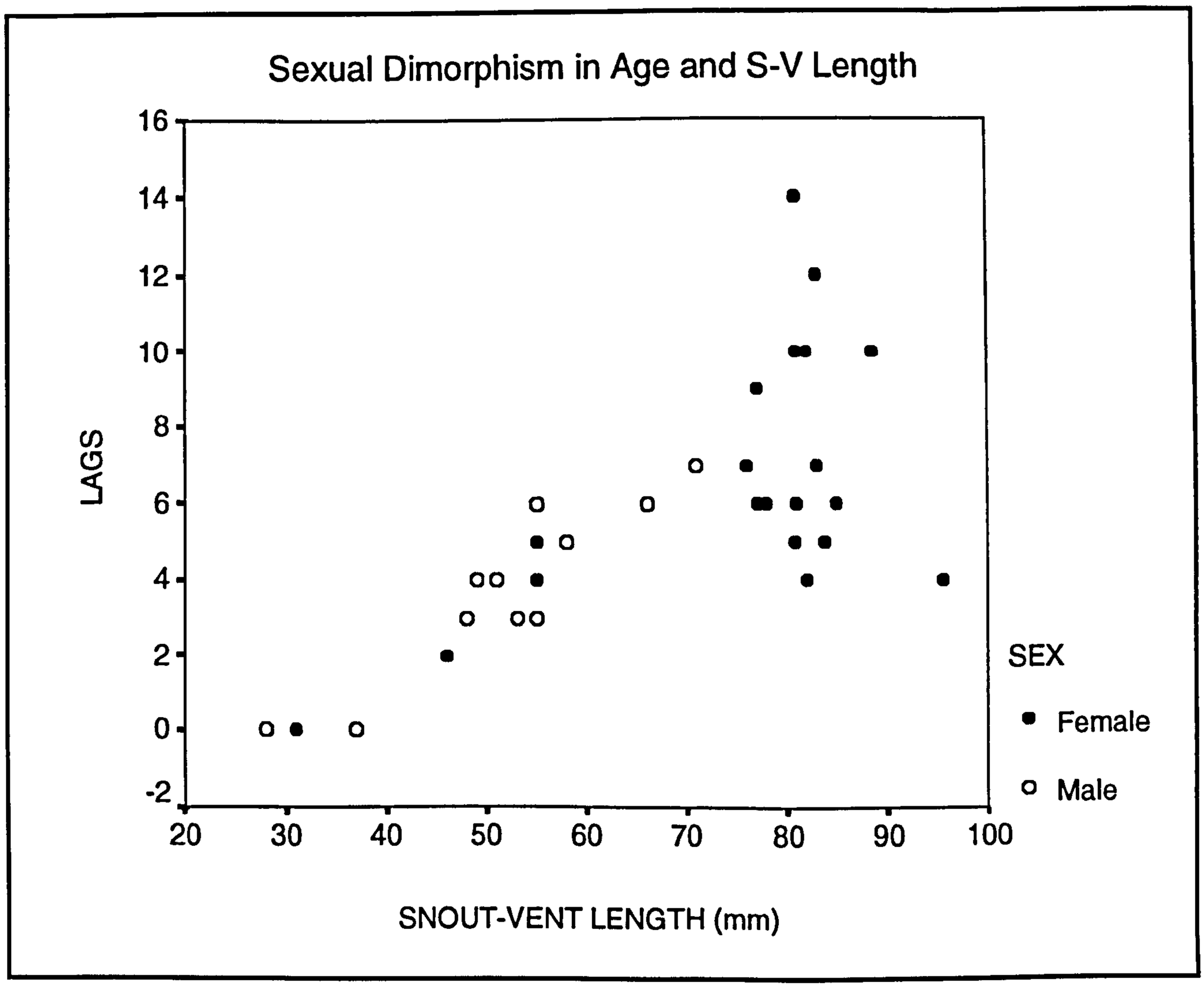


Figure 3-3

Snout-vent length (mm) and age ranges (LAG) for male and female samples. Black dots– females; open circles – males.

Sexual Dimorphism

The degree of sexual dimorphism in snout-vent length emphasized the importance of assessing the differential effects of sex on osteology and of assessing the effects of age separately for each sex. Sexual dimorphism in osteology was initially investigated for each character in turn using a two-sample t-test for data standardized against snout-vent length. Only significant results are listed in Table 3-1.

Character number	Character	Female, n=23,mean± s.d.	Male, n=11,mean± s.d.	*p<0.05, **p<0.01, ***p<0.001
1	Skull width with tympanic annulus	2.03±0.15	2.21±1.01	***
1b	Width occipitopetrosal	1.46±0.122	1.64±0.14	**
2	Length skull	1.84±0.18	2.08±0.22	**
4	Width nasal	0.66±0.09	0.74±0.05	**
5	Length nasal	0.37±0.04	0.43±0.04	***
17a	Length frontoparietal	1.3±0.14	1.47±0.15	*
21	Dist. between frontoparietal and foramen magnum	0.48±0.09	0.58±0.07	**
29	Width foramen magnum	0.58±0.11	0.72±0.08	***
39	Diameter optic foramen	0.05±0.04	0.108±0.04	**
44	Length tympanic annulus	0.39±0.04	0.47±0.02	***
50	Length premaxillary	0.22±0.02	0.23±0.02	*
51	Length maxillary	0.67±0.07	0.76±0.07	**
52	Length dental ridge	0.56±0.67	0.65±0.06	***
53a	Length maxillary arch	0.5±0.6	0.58±0.08	**
53b	" " " including pars facialis	0.57±0.07	0.66±0.09	**
54	Premaxillary tooth count	0.14±0.97	0.18±0.04	*
55	Maxillary toothcount	0.397±0.14	0.55±0.07	***
63	Distance between base of anterior pterygoid rami	0.89±0.18	1.0±0.06	*
74	Length 1st transverse process	1.16±0.11	1.33±0.11	***
77b	Width sacral diapophysis	1.21±0.13	1.29±0.08	*
83	Length tibo-fibula	2.49±0.12	2.6±0.11	**
84	Length tibiale-fibulare	1.35±0.06	1.47±0.1	**
85	Length 2nd metatarsal	2.8±0.16	3.1±0.14	***
86	Length humerus	1.25±0.05	1.45±0.09	***
87	Radio-ulnare length	1.96±0.18	1.18±0.14	***
88	Length 2nd metacarpal	1.24±0.09	1.35±0.09	**
90	Length ischium	0.52±0.056	0.45±0.05	**
95	Medial distance between clavicle and coracoid	1.27±0.1	1.41±0.16	*
98	Zonal plane/coracoid angle	0.48±0.2	0.73±0.3	*

Table 3-1

Univariate t-test results for sexual dimorphism of osteological characters listed in Appendix 1, mean and standard deviation values are given for each character (only characters that vary significantly between sexes are listed). Asterisks refer to the degree of sexual dimorphism. Characters have been standardized against snout-vent length.

Principal component analysis was subsequently conducted on data for comparison with t-test results (Table 3-1). PCA1 comprised almost exclusively of coefficients of the same sign and magnitude, expressing size variation in the sample, and accounting for 80.5% of the total variation explained by the analysis. The exceptions, variables whose coefficients were negative or of a different magnitude, are listed in Appendix 2, and represent characters that are either inversely related to, or vary independently of s-v (snout-vent) length.

Remaining components, PCA 2-8, expressed shape variation and were analyzed using DFA (grouping variable: sex) to identify the primary form of shape variation between sexes. DFA1 accounted for 100% of the sex-related variation identified by the analysis, and sexual dimorphism was calculated as being significant (Rao's F=10.28, d.f.=1,32, p=0.001). Correspondingly, the DFA rate of correct classification of cases is high (82.4% of original grouped specimens). DFA 1 coefficients indicated that PCA4 explained most of this size-independent sexual dimorphism (Table 3-2). Reference to character weightings for PCA4 indicated that characters listed in Table 3-3 accounted for most of the observed sexual dimorphism. These were highly congruent with results from t-tests. PCA4 and 5, the two most important discriminators, were plotted against one another and showed moderate shape distinction between sexes (Figure 3-4).

Coefficients for DFA (on PCA factors 2-8)	
	DFA 1
PCA factor 2	-0.0296
PCA factor 3	-0.0225
PCA factor 4	**0.9095**
PCA factor 5	-0.409
PCA factor 6	0.251
PCA factor 7	-0.337
PCA factor 8	-0.034

Table 3-2

Coefficient scores for Discriminant Function Analysis on Principal Component Analysis factors 2-8 (grouping variable: sex), showing PCA4 as accounting best for size-independent sexual dimorphism in osteological characters listed in Appendix 1.

Character number	Character	PCA, factor 4 component scores
39**	Length optic foramen	0.463
63*	Distance between base of anterior pterygoid ramus	0.381
55***	Number of maxillary teeth	0.341
102	Ultimate phalanx length, metacarpal I	-.0315
54*	Number of pre-maxillary teeth	-.0283
52***	Length dental ridge	0.247
40	Retractor bulbi muscle scars	0.229
99	Proximal pre-hallical element length	-0.228
44***	Tympanic annulus length	0.206
62	Inter-otic gap	0.199
90**	Ischium length	-0.193
19	Width of frontoparietal at level of occipitopetrosal	-0.154
24	Length frontoparietal posterior from pineal foramen	-0.145
101	Penultimate phalanx length, metacarpal I	-0.144
17b	Distance between frontoparietal and foramen magnum	-0.139
51**	Length of maxillary	0.134

Table 3-3

Component scores for PCA4, the factor accounting for most of the observed sexual dimorphism. Scores correspond to the relative magnitude of sexual dimorphism expressed by that character. Asterisks adjacent to character numbers refer to corresponding t-test significance levels for sexual dimorphism (see Table 3-1).

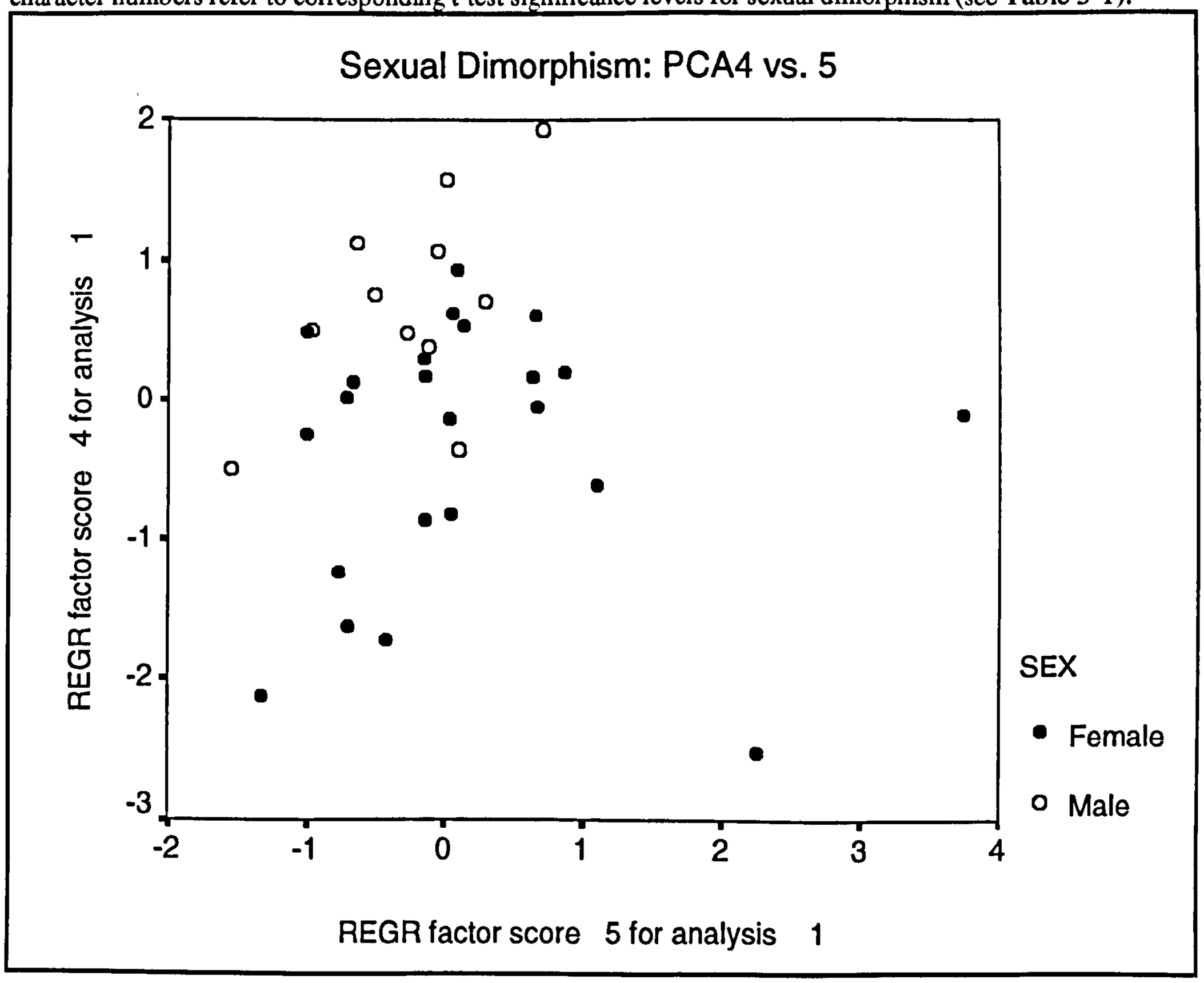


Figure 3-4

Scatter plot of PCA 4 and 5, the factors which explain most of the observed sexual dimorphism in osteology. Black dots – female; open circles – male. (REGR= Principal component analysis regression)

Age-related osteological variation

It was necessary for analysis of age-related variation to place specimens into age categories despite this compromising the sensitivity of results. Sample-size dictated category ranges. Animals were placed into four groups, each spanning three LAG, except in the case of the youngest and oldest groups, which included specimens of one additional LAG; groups were numbered 1-4 and represent 0-3, 4-6, 7-9 and 10-14 LAG respectively. Sexes were initially pooled in order to maximize sample sizes for analysis. Sexual-dimorphism in age-related variation was examined separately.

One-way analysis of variance was conducted on each variable in turn, standardized against snout-vent length. Significant results are listed in Table 3-4. Post-hoc Tukey-tests were used to

calculate where this variation lay (groups preceding brackets varied significantly from those enclosed in brackets). Except where italicized, character size, relative to S-V length, decreased with age.

Character number	Character	F	*p<0.05, **p<0.01, ***p<0.001	Tukey
1	Skull width (including tympanic annulus)	15.31	***	1(2,3,4)
1b	Skull width	20.4	***	1(2,3,4)
2	Skull length	13.08	***	1(2,3,4)
4	Maximum width nasal	20.71	***	1(2,3,4), 2(4)
5	Length nasal	5.13	***	1(2,3,4)
12	Nasal/ septmaxillary anterior gap	12.35	**	1(3,4)
17a	Frontoparietal length	4.14	***	1(2,3,4)
17b	" " to occipitopetrosal base	10.69	*	1(4)
19	Width frontoparietal at association with occipitopetrosal	0.1	***	1(2,3,4)
21	Distance from frontoparietal to occipitopetrosal	19.15	***	1(2,3,4)
24	Frontoparietal length posterior from pineal foramen	8.7	***	1(2,3,4)
29	Width foramen magnum	18.2	***	1(2,3,4)
30	Depth foramen magnum	10.59	***	1(2,3,4)
39	Diameter optic foramen	13.28	***	1(2,3,4)
50	Length pre-maxillary	6.11	*	1(4), 2(4)
51	Length maxillary	9.96	***	1(2,3,4)
52	Length dental ridge	10.4	***	1(2,3,4)
53a	Depth maxillary arch from tooth row	13.53	***	1(2,3,4)
53b	" " " including pars facialis	12.57	***	1(2,3,4)
54	Number of pre-maxillary teeth	16.04	***	1(2,3,4)
55	Number of maxillary teeth	15.08	***	1(2,3,4)
71	Atlas width	12.5	***	1(2,3,4)
73	*Vertebral column length*	*3.53*	*	*1(4)*
74	Width of first transverse process	6.91	**	1(2,4)
75	Width at second transverse process	8.03	***	1(2,3,4)
77	*Sacral diapophysis length*	*6.4*	**	*1(2,3,4)*
77b	Width sacral diapophysis	6.18	**	1(2,3,4)
78	Urostyle length	4.17	**	1(4)
83	Length fibio-tibia	3.05	*	1(4)
84	Length fibiale-tibulare	5.67	**	1(3,4)
85	Length second carpal digit	9.83	***	1(2,3,4)
90	*Length ischium*	*6.98*	**	*1(2,3,4)*
98	Zonal- plane/ coracoid angle	14.13	***	1(2,3,4)
99	Proximal pre-hallux length	3.24	*	1(4)
101	Penultimate phalanx length, metatarsal I	6.79	**	1(2,3,4)

Table 3-4

Degrees of freedom- 3,20. Characters listed by number in Appendix 1. Anova results showing significance levels (asterisks) for the effects of age on osteology (sexes pooled). Characters in age-groups preceding brackets varied significantly from those in groups enclosed by brackets (groups 1-4: 0-3, 4-6, 7-9, 10-14 LAG). F values indicate the degree of variation between age groups.

Multivariate analysis was subsequently conducted to identify the effects of age on osteology for the pooled-sex sample. All principal component factors except for PCA1, the size-factor, were used in DFA analysis (grouping variable: age-category, to assess the degree of shape variation between age categories). DFA1 accounted for 88% of the total variation explained by the analysis, its coefficients indicating that PCA2 explained most of the size-independent variation in age (Table 3-5).

Standardized Canonical Discriminant Function Coefficients	
	Function 1
PCA factor 2	**0.816**
PCA factor 3	0.605
PCA factor 4	0.350
PCA factor 5	-0.019
PCA factor 6	-0.116
PCA factor 7	0.052
PCA factor 8	-0.241

Table 3-5

Coefficient scores for Discriminant Function Analysis on Principal Component Analysis factors 2-8 (grouping variable: age category), showing PCA2 as best accounting for size-independent sexual dimorphism in osteological characters listed in Appendix 1.

Cross-reference to character weightings for PCA2 demonstrated that characters listed in Table 3-6 accounted for most of this variation.

Character number	Character	PCA 2 component scores
54* **	Number of pre-maxillary teeth	0.823
98***	Zonal plane/coracoid angle	-0.596
55* **	Number of maxillary teeth	0.595
39* **	Length of optic foramen	0.369
63	Distance between base anterior pterygoid rami	-0.293
72	Depth of atlas	-0.268
103	Pre-pollex length	0.221
101 **	Penultimate phalanx length, metacarpal I	0.214
62	Inter-otic gap	-.0176
19* **	Width frontoparietal at level occipitopetrosal	-0.173
40	Retractor bulbi muscle scars	0.150
20a	Length fp and from point of assoc with occ. pet.	-0.148
34	Max width vomer	0.141
21***	Length between fp and foramen magnum	0.136
99*	Proximal pre-hallical element length	0.135

Table 3-6

PCA2 component scores corresponding to characters showing greatest age-related variation (pooled-sex). Component scores correspond to the magnitude of difference between age-groups. Asterisks refer to corresponding one-way analysis of variance probability levels for each character listed in Table 3-4 and bold type refers to characters that have been shown to demonstrate sexual dimorphism (see Table 3-1). Abbreviations: fp: frontoparietal, occ. pet.:occipitopetrosal.

There was a high degree of congruence between results from univariate and multivariate methods. Over 50% of the variables listed in Table 3-6 as varying with age are also listed in Table 3-3 as being sexually dimorphic, more specifically, 4 of the first 5 characters are shared by each character-list.

Scores for PCA2 and 3 (the second highest coefficient) were plotted against one another, but did not illustrate a clear distinction between age-categories (Figure 3-5). This reflects the poor ability of DFA to classify specimens correctly to age-group (57.6% original groups correctly classified). Analysis of variance, comparing mean DFA factor scores between different age-groups indicated a significant, if weak, difference between groups 2 and 4 only (Tukey's test: F=3.99, d.f.=3, 30, p= 0.017). Furthermore, mean scores for each successive age-group were not sequential, demonstrating that although a number of characters varied in an age-related manner, overall age-related shape variation was not linear.

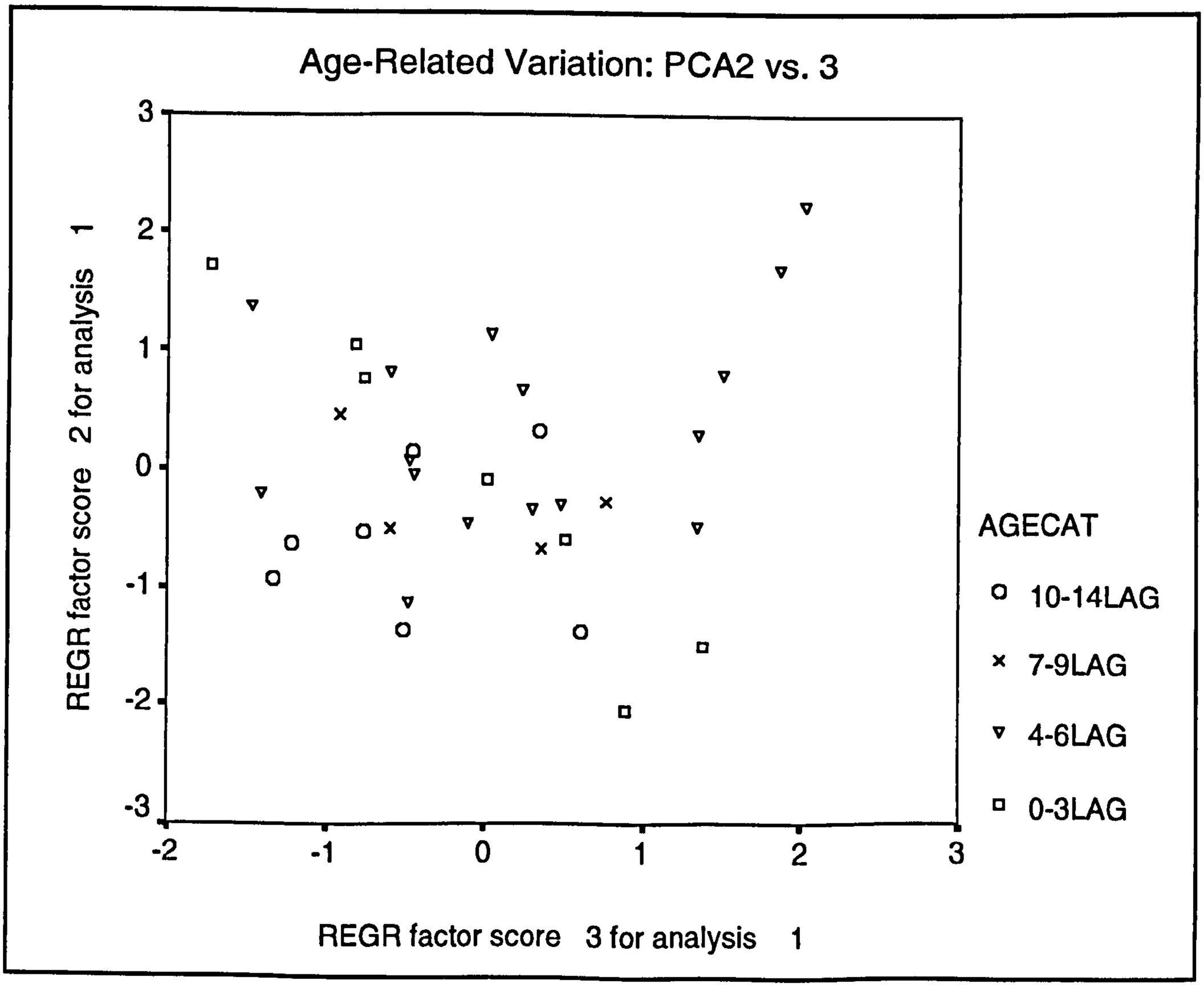


Figure 3-5

Scatter graph, PCA2 and 3, representing factors that best explain age-related osteological variation (pooled-sex). Age categories 1-4: 0-3, 4-6, 7-9, 10-14LAG.

Evidence of sexual dimorphism in age-related osteological variation

The high degree of sexual dimorphism in snout-vent length and congruence between results from both sex- and age-related variation studies (Table 3-3 and Table 3-6) suggested a need to analyze age-related variation separately for each sex. Sample-size restrictions meant that statistical analysis was only possible for females however, and necessarily involved a reduction in the number of age-categories. The resultant reduction in sensitivity of the analysis was traded for maintaining statistical accuracy. Sexual-dimorphism in age-related variation was subsequently inferred from differences between results from pooled-sex and female-only analyses. Category ranges were determined by sample-size. Three categories were used, comprising specimens with 0-4, 5-9 and 10-14 LAG.

Significant results from analysis of variance, comparing shape-variation between age groups in the female sample are presented in Table 3-7. Size of all listed characters standardized against snout-vent length decreased with age.

Character number	Character	F	*p<0.05, **p<0.01, ***p<0.001	Tukey
1	Width skull including tympanic annulus	5.86	*	1(3)
1b	Width occipitopetrosal	7.16	**	1(2,3)
2	Length skull	10.27	**	1(2,3)
4	Width nasal	7.56	**	1(3)
5	Length nasal	14.36	***	1(2,3)
12	Distance between anterior septmaxilary and nasal	4.39	*	
17a	Length frontoparietal (fp)	7.34	**	1(2,3)
17b	Length from anterior fp to foramen magnum	4.83	*	1(3)
19	Width frotnoparietal at level of association with occipitopetrosal	17.16	***	1(2,3)
21	Distance between frontopa-rietal and foramen magnum	34.32	***	1(2,3)
24	Length frontoparietal post-erior from pineal foramen	11.59	***	1(2,3)
28	Width foramen magnum	24.13	***	1(2,3)
30	Depth foramen magnum	9.92	**	1(2,3)
39	Diameter optic foramen	7.48	**	1(2,3)
42	Length columella	5.46	*	1(3)
50	Length premaxillary	4.67	*	1(3)
51	Length maxillary	6.37	**	1(3)
52	Length dental ridge	6.7	**	1(3)
53a	Length maxillary arc	8.78	**	1(3)
53b	" " " including pars facialis	8	**	1(2,3)
54	Premaxillary tooth count	16.89	***	1(2,3)
55	Maxillary tooth count	31.55	***	1(2,3)
71	Atlas width	6.75	*	1(3)
74	Length 1st transverse process	6.64	**	1(3)
75	Length 2nd transverse process	4.66	*	1(3)
77b	Length sacral diapophysis	5.23	*	1(3)
84	Length fibiale tibulare	3.86	*	1(3)
88	2nd metacarpal length	3.74	*	1(3)
91	Width ant acetabular	4.47	*	1(3)
98	Zonal/coracoid angle	8.95	**	1(2,3)
99	Proximal pre-hallux length	7.17	**	1(3)
101	Penultimate phalanx length, metatarsal II	5.56	*	1(2,3)

Table 3-7

Degrees of freedom, 2,20. Characters listed by number in Appendix 1. Anova results showing significance levels (asterisks) for the effects of age on osteological variation (female-only sample). Characters in age-groups preceding brackets varied significantly from those in age-groups enclosed by brackets (groups 1-3: 0-4, 5-9, 10-14 LAG). F values indicate the relative degree of variation between age-groups.

Size-out DFA of age variation (grouping variable: age-category) in females was carried out. DFA1 accounted 74% of the total age-variation explained by the analysis, and 69.6% of original cases were correctly classified. Coefficient scores for DFA1 are greatest for PCA7. Of the corresponding PCA characters coefficients (Table 3-8), the four most heavily weighted characters match four of the five most heavily weighted characters in the pooled-sex analysis, together accounting for a high proportion of total character weightings for this function.

Character number	Character	PCA 7 component scores
39**	Diameter optic foramen	0.456303
63	Distance between base anterior pterygoid ramus	0.362407
54***	Pre-maxillary tooth count	0.244467
98**	Zonal plane/ coracoid axis	0.24121
87	Length radio-ulnare	-0.21731
30**	Depth foramen magnum	0.174065
22	Distance from tip foramen magnum to pineal foramen (pineal foramen)	0.171766
4**	Maximum width nasal	0.153159
74**	Length first transverse process	0.145578
40	Inter-rtbs (retractor bulbi muscle scar) gap	-0.13823
62	Inter-otic gap	-0.13555
24***	Frontoparietal length posterior from pineal foramen	-0.13068
43	Length squamosal arm	-0.12881
12*	Ant gap between nasal/ septomaxillary	-0.12609
77b*	Width sacral diapophysis	-0.12536

Table 3-8

PCA7 component scores corresponding to characters showing greatest age-related variation (female sample). Component scores correspond to the magnitude of difference between age-groups. Bold type- characters occurring in results from poled-sex and female-only studies (see Table 3-6), asterisks refer to corresponding probability levels from t-tests (Table 3-7).

Significant results were returned from analysis of variance, carried out to compare mean DFA scores between different age-groups ($F=5.56$, d.f.=2,20, $p=0.012$), groups varying sequentially with age.

Size related osteological variation

Size-related variation was also analyzed for females only, following the same procedure as that used in the preceding, age-related variation study. Specimens were placed into 3 size-categories, dictated by sample size. These were 30-76, 77-82, and 83-100mm.

One-way analysis of variance was carried out on each variable to compare changes in relative character size (standardized against snout-vent length) between each size-category. Characters varying significantly are presented (Table 3-9). Tukey-tests were carried out to identify where this variation lay (groups preceding brackets varied significantly from those enclosed in brackets). Relative size for each character became smaller with an increase in s-v length.

Character number	Character	F	*p<0.05, **p<0.01, ***p<0.001	Tukey
1	Width skull with tympanic annulus	4.39	*	1(2,3)
1b	Width occipitopetrosal	4.28	*	1(2,3)
2	Length skull	5.3	*	1(2,3)
4	Width nasal	4.58	*	1(3)
5	Length nasal	4.1	*	1(3)
12	Distance between anterior septmaxillary and nasal	5.82	*	1(2,3)
17a	Length frontoparietal	4.76	*	1(2,3)
19	Width frontoparietal at level of association with ocipitopetrosal	5.12	*	1(2,3)
21	Distance between frontoparietal and foramen magnum	6.72	**	1(2,3)
24	Length frontoparietal posterior from pineal foramen	6.38	**	1(2), 2(3)
29	Width foramen magnum	8.29	**	1(2,3)
30	Depth foramen magnum	4.59	*	1(2,3)
44	Length tympanic annulus	6.32	**	1(2,3)
51	Length maxillary	5.2	*	1(2,3)
52	Length dental ridge	7.94	**	1(2,3)
53a	Length maxillary arch	6.66	**	1(2,3)
53b	" inc. pars facialis	6.43	**	1(2,3)
54	Premaxillary tooth count	13.31	***	1(2,3)
55	Maxillary tooth count	19.45	***	1(2,3)
84	Fibiale tibulare length	3.72	*	1(2,3)
85	Length 2nd metatarsal	4.59	*	1(2,3)
88	Length 2nd metacarpal	6.64	**	1(2,3)
98	Zonal/coracoid angle	4.42	*	1(2,3)

Table 3-9

Degrees of freedom, 2,20. Characters listed by number in Appendix 1. Anova results showing significance levels (asterisks) for the effects of size on osteological variation (female-only sample). Characters in size-groups preceding brackets varied significantly from those in groups enclosed by brackets (groups 1-3: 30-76, 77-82, 83-100mm).

Size-out DFA analysis (grouping variable: size-category) of allometry was carried out for the female sample. Coefficients from DFA1 showed that PCA2 explained most of the size-independent variation in shape between different size-groups (Table 3-10). DFA1 accounted for 81.7% of the total variation explained by the analysis.

Standardized canonical Discriminant Function Coefficients	
PCA factor 2	**0.778**
PCA factor 3	-0.089
PCA factor 4	-0.351
PCA factor 5	0.212
PCA factor 6	0.484
PCA factor 7	-0.016
PCA factor 8	-0.738

Table 3-10

DFA (on PCA 2-8) coefficients. Grouping variable: size-category. Coefficient scores for DFA on PCA factors 2-8 (grouping variable: size category), showing PCA2 as accounting best for allometric osteological variation.

Weightings of these discriminant function coefficients were similar to those explaining variation in age in the pooled-sex analysis (Table 3-5), but differed from results of the female-only age analysis. Despite these differences, cross-correlation with PCA character weightings reveal that the characters showing greatest variation between size-groups were the same as those identified by the pooled-sex analysis of age-variation, and matched 4 of the first 5 from the female age-variation study.

DFA was able to classify 78.3% of original cases correctly. Results from analysis of variance carried out to compare mean DFA scores for different age-groups showed groups to be significantly different from each other ($F=8.98$, d.f.=2, 20, $p=0.002$). A Tukey-test carried out to identify where this variation lay showed, however, that mean DFA scores were not sequential for successive size-groups.

Differences in the explanatory power of DFA1 (sex: 100% cases correctly classified, pooled-sex age: 88%, female-only age: 74%; female-only size 81.7%) and the relative magnitude of coefficients listed for this function reflect variation in relative discriminatory importance of each set of characters for each analysis. Likewise, the slight variation observed in PCA character coefficients for each analysis corresponds to variation in the order of importance of each character for discrimination between groups.

Qualitative intra-specific variation

A number of additional, qualitative observations were made, and variable characters scored accordingly. Data were ranked by sex, size and age to assess sexual dimorphism and to determine whether progressive character state changes were more strongly size- or age-related.

The sequence of appearance of calcification of rostral cranial elements was more strongly size- than age-related. Appearance of 'element A' (*sensu* Smirnov, 1994) in the anterior nasal cavity and calcification of the planum-antorbitale appeared first, becoming extensive in larger specimens and was followed by calcification of the septum-nasi and finally of cartilage of the alary processes. Calcification of suprascapular cartilage and laryngeal cartilage in the case of males, as for cranial

elements, was more strongly size- than age-related. There was no sexual dimorphism in the timing or sequence of calcification. The position of parasagittal crests, relative to each, other was also variable. Whilst remaining parallel throughout life, they migrated medially with growth. This migration appeared to be indeterminate, crests forming a highly pronounced ridge in the largest females, being more strongly correlated with size than age. Frontoparietal shape was directly related to the degree of crest development, and was more highly correlated with specimen size than age. The cultriform process in some of the largest females was uncharacteristically short and did not reach as far as the partes palatina. Development of ventral occipitopetrosal protrusions and the crista parotica seemed to be indeterminate and was more closely related to size than age, displaying no evidence of sexual dimorphism. Fusion between adjacent cranial elements was rare, and occurred only between the sphenethmoid and occipitopetrosal in a few of the larger females, irrespective of their age, and in one of the largest male specimens. Asymmetry of sacral diapophyses around the transverse plane was strongly size-related, and as such was more extreme in the largest females than in any of the male specimens. The degree of neural arch imbricateness was also more strongly size- than age-related, becoming highly ornate in larger females, generally developing pronounced, single, posteriorly directed spinous processes.

The scapula was cleft in some specimens, whilst partes acromialis and glenoidialis in other specimens were fully fused. An intermediate state of partial fusion occurred in others, in which distal margins of partes were fused, enclosing a foramen between proximal margins. Fusion appeared to be more strongly size-related in males and age-related in females.

Characters showing least intra-specific variation

As well as highlighting the most variable osteological characters, these analyses have also served to identify those showing isometric growth and varying least with age and sex. These are given below in list form. Those in bold type are considered to characterize the taxon, varying considerably from all others examined during the course of the present study:

Frontoparietal- anterior width, relative position of pineal foramen, length anterior from its point of association with the occipitopetrosal, width at point of association with occipitopetrosal.

Sphenethmoid- anterior width and width at point of association with occipitopetrosal, **distance between rtbs** (retractor bulbi muscle scars), relative width of (edentate) vomer.

Other: Distance between base of anterior pterygoid rami and between otic plates, columella length, **clavicle medial width**, scapula length, relative lengths of proximal and distal prehallux elements, iliac shaft and urostyle lengths.

XENOPUS LAEVIS LAEVIS- OSTEOLOGICAL DESCRIPTION

Although *X. laevis* has been widely used in several disciplines of science since the 1930's, and has been referred to in almost all accounts which deal with osteology in the genus, no single account exists which describes *X. laevis* osteology in its entirety. A number of accounts deal in detail with, or mention certain aspects of osteology in the taxon (e.g. Paterson [1939] described the cranium in an account that also describes nerves, muscle and cartilages. Reumer [1985] and Smirnov [1994]: selected features of the cranium. Griffiths [1954]: frontoparietal. Jurgens [1971]: nasal region. Sassoon and Kelley [1986]: larynx. Van Dijk [unpublished]: pre- and post-zygapophyses. Palmer [1960] and Emerson [1979, 1982]: ilio-sacral articulation. Parker [1934]: distal prehallux element. Fabrezi [1993]: tarsal elements. [Fabrezi, 1996]: carpal elements. [Fabrezi, 2001]: prepollex and prehallux). Several cladistic analyses have also included information pertaining to osteology in *X. laevis* (e.g. Baez [1981], Trueb and Cannatella [1986], Cannatella and Trueb [1988], Baez and Trueb, [1997]). The following account provides the first complete description of osteology in this taxon. Illustrations are of a 12 LAG female specimen and should be compared with illustrations 1,2,3,5 and 6 in chapter 2, which are of a 5 LAG female specimen.

Cranium

Frontoparietal

This hourglass-shaped element is as wide anterolaterally as at its anterior-most point of association with occipitopetrosals (Illustration 3-1). The element assumes an approximately square caudal profile with pronounced posterolateral alae. An obtusely angled apex forms the anterior margin. There is no dorsal skull table *per se*, parasagittal crests meeting to form a medially raised crest, accentuating the great length of this element. This medial ridge extends posteriorly almost to the level of the foramen magnum, and anteriorly to the level of anterolateral corners of the element. Braincase walls, lateral to this crest, are straight, extending to reach the sphenethmoid laterally, and occipitopetrosal caudally, except in the region of cerebral hemispheres, where the braincase is bi-laterally convex. The pineal foramen is positioned within the anterior quarter of the element.

Sphenethmoid and vomer

Slightly wider anteriorly than at its point of association with the occipitopetrosal, this element is occasionally waisted (Illustration 3-2). In most specimens, the element is evenly curved in transverse profile, though in some larger specimens, a ventral keel develops. The anterior margin bears a pair of laterally-displaced notches representing orbitonasal foramina. Rtbs are small and are either separated by a narrow gap or form a continuous trough across the base of the element. Optic foramina occur within rtbs, are small and laterally-displaced, the aperture invisible in ventral view.

An uneven crest extends between posterior margins of rtbs in some specimens. The element lacks posterolateral alae, narrowing abruptly beyond posterior margins of rtbs to form an elliptic caudal process that almost reaches the level of the foramen magnum. The moderate sized vomer is usually azygous, cylindrical in shape, may occasionally be paired, and is always edentulous. This element is positioned over the base of the cultriform process, which was mistaken for the vomer by Paterson (1939).

Occipitopetrosal

Anterior margins are perpendicular and posterior margins oblique with respect to the zonal axis, converging on well-developed, long cristae parotica (Illustration 3-1, Illustration 3-2). Medial margins are partly fused around the wide foramen magnum. Dorsal crests are highly pronounced, giving the remainder of the element the appearance of concavity, accentuated by the great length of cristae parotica. Eustachian canals are deep and the ridges that form their posterior margins directed posteromedially towards the widest point of the caudal sphenethmoid process. Paired, caudally-directed processes associated with this ridge are particularly long and slender.

Pterygoid

Anterior and lateral rami lack any distinguishing features (Illustration 3-1, Illustration 3-2). Both proximal and distal flanges of this ramus are moderately slender, the latter only slightly curved. The otic plate is asymmetrically bifid, the smaller ala tapering to a point and extending anteromedially around the pterygoid knob, whilst the larger of the two is extremely broad with an evenly curved medial margin, and posterior margin oriented at an angle perpendicular to the main body axis. The inter-otic plate gap is very narrow.

Tympanosquamosal

The annulus is round, bearing a small posteroventral ala that supports the columella at its pivot point (Illustration 3-1). A semi-robust squamosal extends towards the distal flange of the anterior pterygoid ramus, with which it associates dorsally or posteriorly by means of an expanded zygomatic ramus, overlapping with the flange slightly in some specimens. The association between squamosal and tympanum is marked by a deep cleft. A short, robust stalk forms the base of the annulus, and does not overlap with occipitopetrosals. The columella is long, narrow and widest at its base, the pars interna plectra. Length anterior and posterior from its pivot point with the annulus is equal.

Maxillary Arch

The maxillary arch is remarkably short, rarely reaching beyond a quarter of the way across the orbit (Illustration 3-2). Dental rows extend along the length of premaxillaries and to the tapered

end of maxillaries. The arch does not extend beyond the anterior-most pterygoid flange. Partes palatina and facialis are slender.

Mandible

As for standard account.

Nasal and Septomaxillary

The rostral nasal extension is often medially scarred, uncleft, and is either parallel-sided or slightly broader rostrally than at its caudal base; there is usually a degree of ventral curvature (Illustration 3-1). Laterally, the semilunar parts of the element bear sub-perpendicularly oriented anterior margins, lack lateral foramina, and are considerably wider than the widest part of the frontoparietal. Septomaxillaries are relatively short, not as steeply oriented as many other taxa, and lack a lateral foramen.

Calcification

Calcification of rostral elements is extensive and, in contrast to some species, includes calcification of element A (*sensu* Smirnov, 1994). Rostral cartilaginous elements are listed in order of propensity for calcification: planum antorbitale, nasal floating elements, rostral nasal and septum, alary (Illustration 3-1).

Degree of fusion

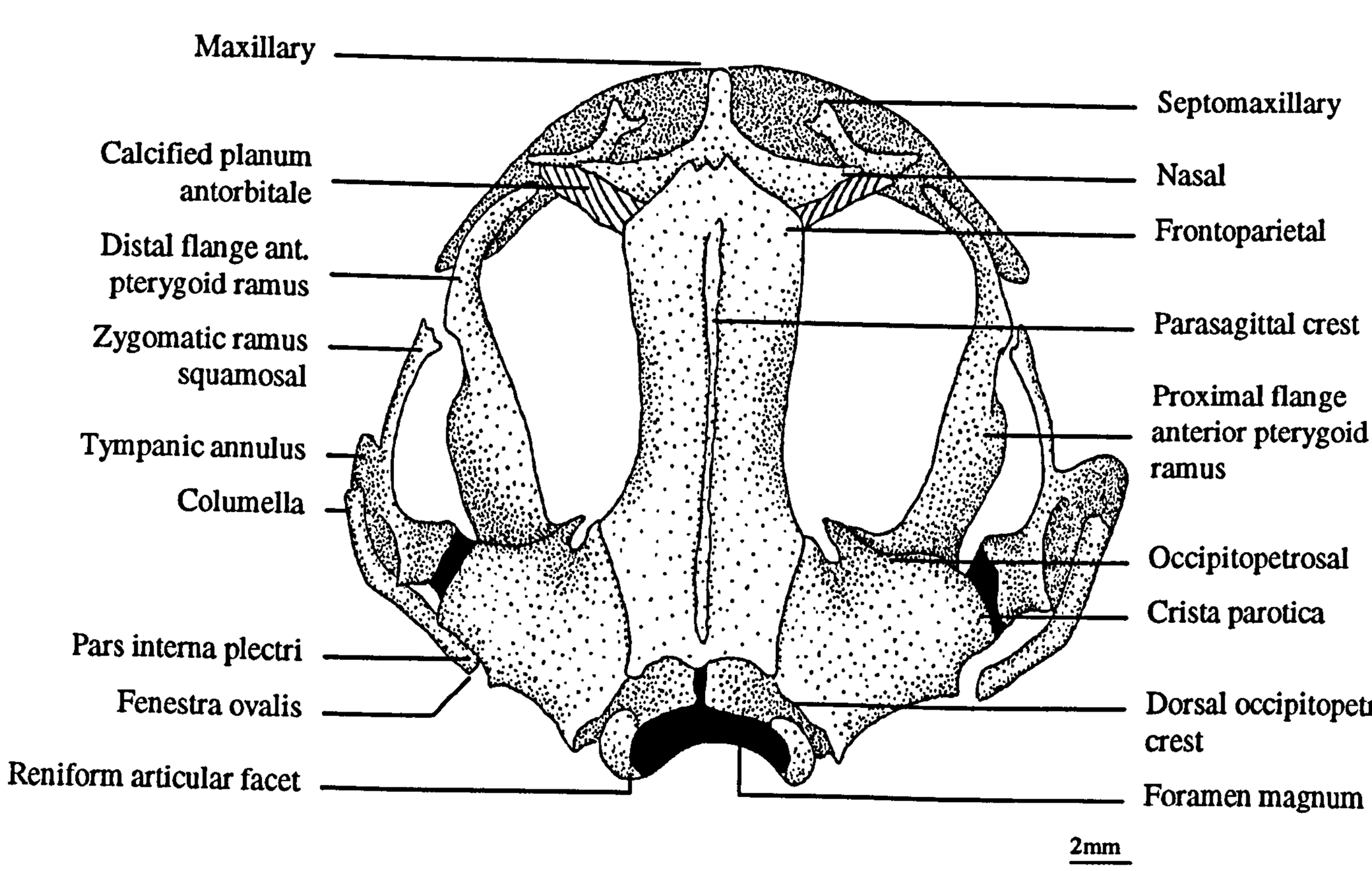
Each of the above elements remains unfused to adjacent cranial components.

Variation, oddities and deformities

Some specimens deviate from the above account in certain respects; the vomer is paired and partly fused to the sphenethmoid in one specimen. Some specimens are characterized by having larger optic foramen and less well-developed parasagittal crests.

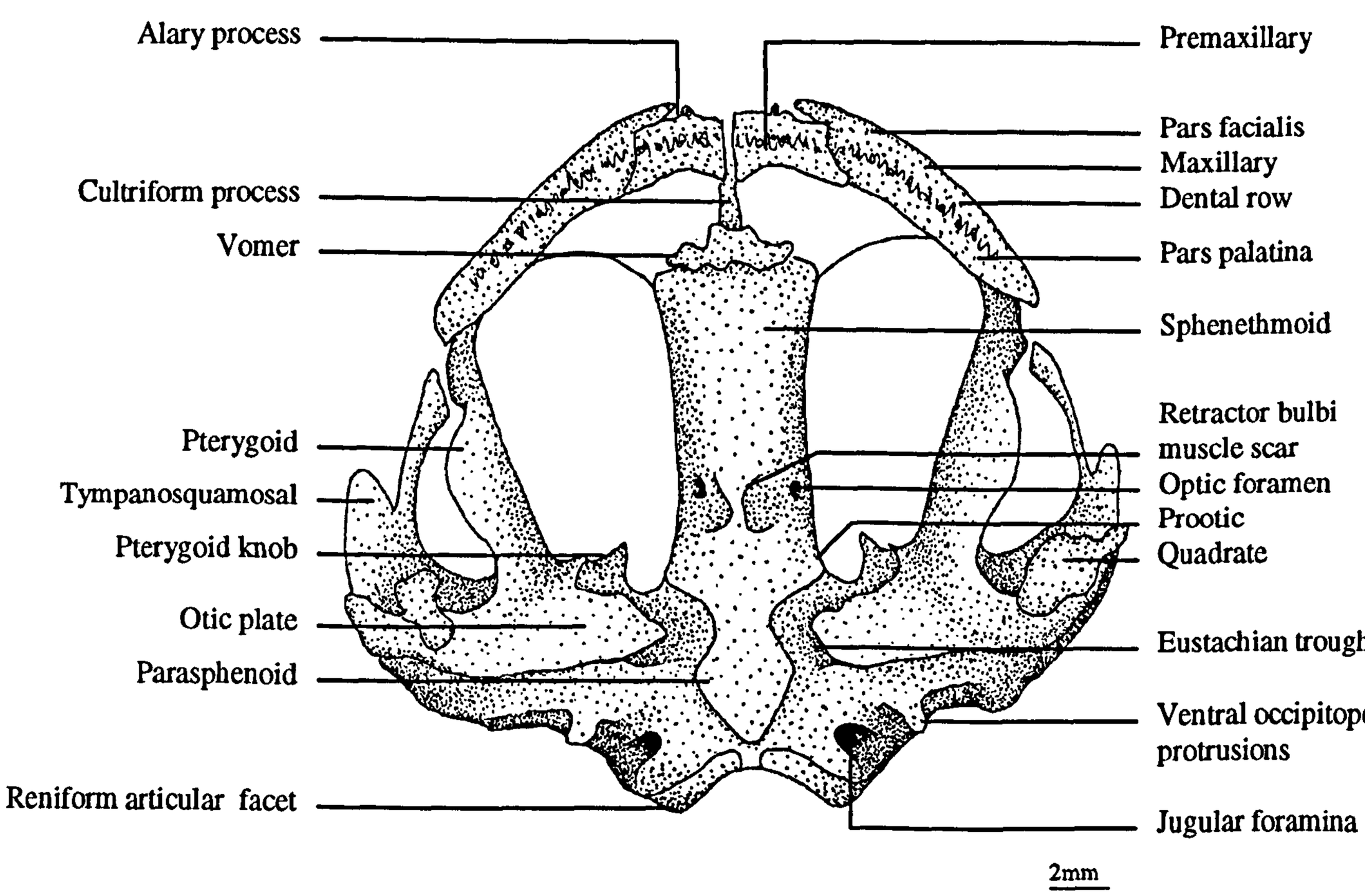
Hyolaryngeal Apparatus

As for account in chapter 2.



Dorsal view skull *X. l. laevis*

Illustration 3-1



Ventral view skull *X. l. laevis*

Illustration 3-2

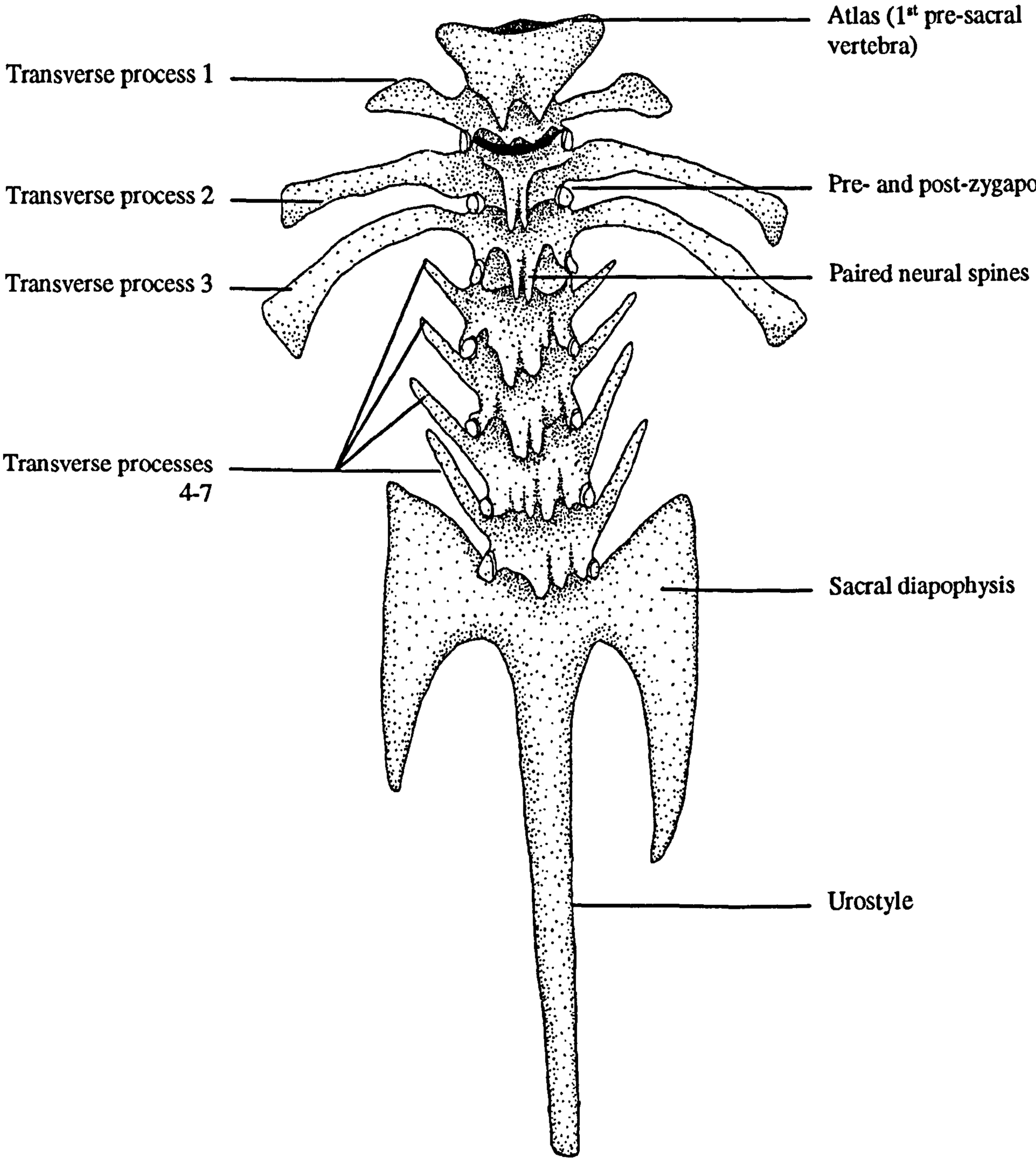
Axial skeleton

Sacral diapophyses and transverse processes are wide relative to vertebral column length (Illustration 3-3). Pre- and post-sacral portions are approximately equal in length. The atlas bears a straight or concave anterodorsal profile narrowing caudally towards a posterior margin that is either irregularly-shaped or bears a pair of neural spines. All subsequent vertebrae are moderately-to-well ornamented, and bear single dorsal spines. Zygapophyses are generally positioned on the outside margin of the column, though may sometimes occur just within the outer profile. The first pair of transverse processes is oriented perpendicularly with respect to the main body axis, with parallel margins along most of their length, tapering distally, each approximately equal in length to the atlas. In some cases these processes are narrow proximally, widening midway along their length and tapering distally. The second pair measures approximately twice the atlas width and leaves the vertebral column at the same angle. Curvature is moderate and mainly proximal. A small degree of distal expansion and ventral curvature is characteristic. The third pair is the longest, curvature and the degree of distal expansion is greater than anteriorly adjacent processes. They measure approximately two and a half times the atlas width.

Ventrally, the atlas bears a convex anterior margin, which is occasionally medially notched. Articular faces between adjacent centra are remarkably broad and spinal foramina separating pedicels correspondingly small. Sacral diapophyses are generally highly asymmetrical around the lateral plane and are parallel-sided. Vestigial processes are generally absent, occurring in only a single specimen.

Variation, oddities and deformities

Vertebrae I and II were fused in a single specimen. The left-hand sacral diapophysis of one specimen was split along a transverse plane.



Dorsal view vertebral column *X. l. laevis*

Illustration 3-3

Anterior appendicular skeleton

Pectoral girdle

Suprascapula and cleithrum are large, overlapping mid-dorsally (Illustration 3-4). A scar marks the site of fusion between pars acromialis and glenoidialis on the short scapula. Clavicles arch anteromedially, their medial margins measuring over three times the lateral width of the element. The angle formed between anterior margins, medially, is acute, whilst posterior margins meet to form an almost straight line. Lateral and medial margins of corâcoids, in contrast, are approximately equal in width, though medial margins may occasionally be slightly wider. These elements are separated from one another medially by a gap that measures approximately the same as their distal width. The mean angle formed between coracoid and zonal plane is low. The prevalence of calcification of epicoracoid cartilage between coracoids and clavicles and also on suprascapula cartilage is high.

Forelimb

As for account in chapter 2. Phalanges are short, relative to those seen in other taxa, and the degree of calcification of carpal elements variable.

Posterior appendicular skeleton

Pelvic girdle

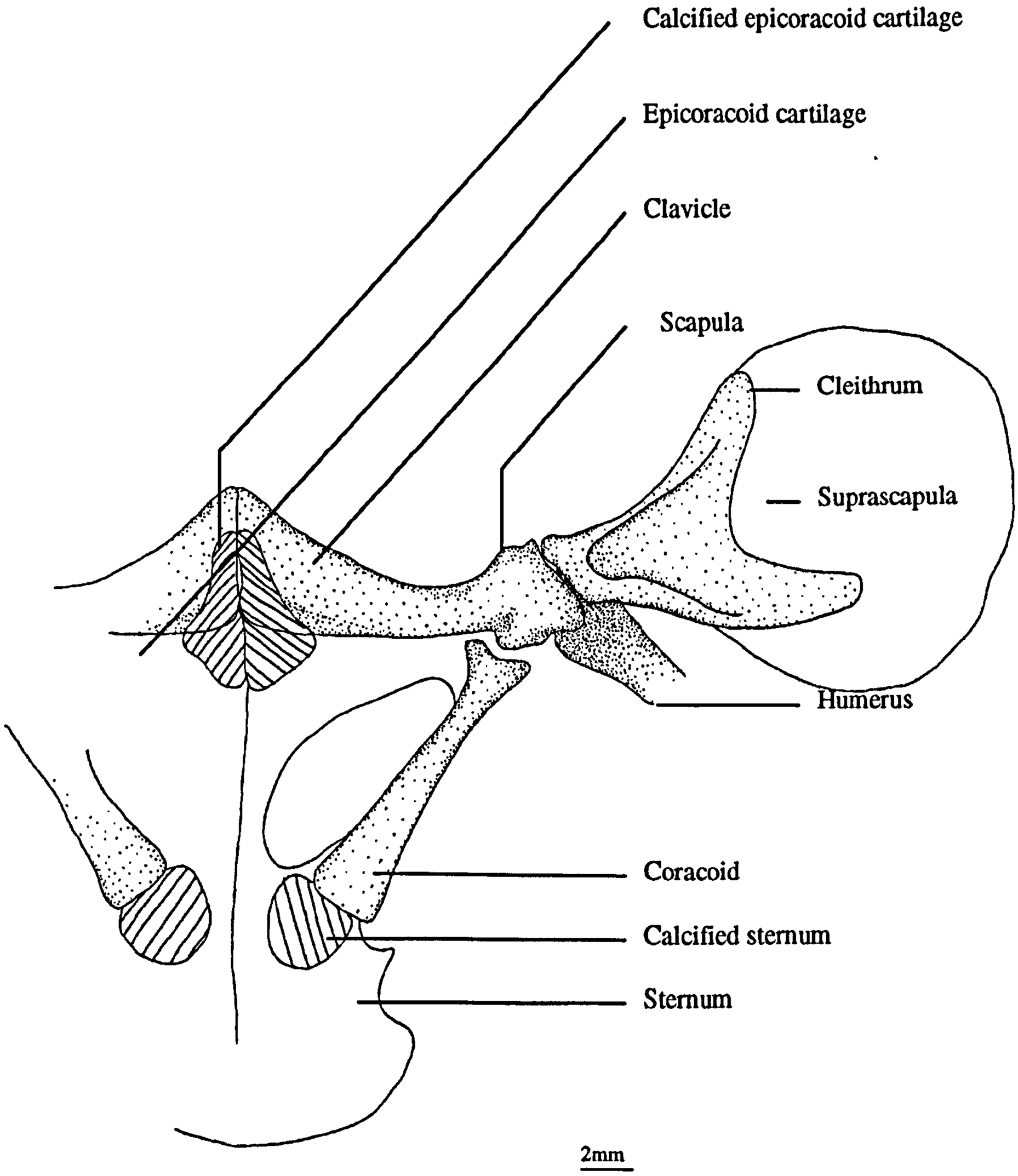
Pelvic elements unite to form a broad, almost square inter-iliac symphysis. Although elements are synchondrotically united, a suture is visible in each.

Hindlimb

As for standard account. The distal prehallux element is parallel-sided, distally curved and measures almost twice that of the proximal element which is oval.

Sexual dimorphism

As for account in chapter 2.



Pectoral girdle *X. l. laevis*

Illustration 3-4

Main features that distinguish X. l. laevis *from other* Xenopus *taxa examined during the course of this study.*

In no other taxon is the posterior margin of frontoparietals so square, nor parasagittal crests medially pronounced as to form a single sagittal crest. The gap separating rtbs is unusually narrow, sometimes meeting to form a trough across the base of the sphenethmoid. Cristae parotica are particularly well-developed and long. Anterior occipitopetrosal margins are oriented perpendicularly, in contrast to the state observed in other non-*laevis* taxa. Eustachian canals are unusually broad, accounting for the caudally positioned ventral occipitopetrosal. The inter-otic gap is smaller than in *X. muelleri*. The tympanic annulus bears a posteroventral process that partially wraps around the pars media plectrum of the columella. The maxillary arch is unusually short, compared with all other non-*laevis* taxa, and has a long dental row. Transverse processes are longer than in any other taxon, the third pair being longer than the second. The suprascapula and cleithrum are considerably larger than non-*laevis* taxa, overlapping mid-dorsally. Medial clavicle margins are greatly expanded, exceeding the degree of expansion seen in other taxa. The relative lengths of prehallux elements distinguish the taxon from *X. muelleri* and *X. largeni*.

DISCUSSION

Sexual dimorphism in snout-vent length for *Xenopus* taxa is widely reported in the literature (Kobel *et al.*, 1996; Tinsley and McCoid, 1996; Measey, 2001). There is no evidence however, of natural variation in age ranges between sexes. Sex-related differences in both size- and age-ranges were pronounced in the present sample. Whilst this could have been a sampling artifact, specimens were collected at random and samples chosen for osteological examination so as to represent the maximum size- and age-range available for each sex. That sexes vary in age-range is consistent with previously conducted studies on *Xenopus* (R. C. Tinsley, pers. com.), indicating variation in life-spans between sexes. Further work is required to corroborate this.

Osteometric data were analyzed so as to identify the main form of intra-specific osteological variation, and to determine, where possible, the cause of this variation, assessing the effects of each of three natural variables in any population, those of sex, age and size. Comparison between results revealed that 4 out of 5 of the most discriminatory characters from each analysis were shared, namely diameter of the optic foramen, distance between bases of anterior pterygoid rami and total number of maxillary and premaxillary teeth. The value representing relative size for each of these characters is greater in males than in females (see Table 3-1), whilst decreasing with age and size. The direction of

sexual dimorphism in these characters is consistent with the male sample representing younger, smaller specimens than the female sample.

Results did not differ significantly between studies to determine sexual dimorphism and age-related variation (pooled-sex and female samples). This implies that the osteological effects of the difference in male and female age-ranges swamped those of sexual dimorphism, the magnitude of age-related osteological variation being greater than that represented by sexual dimorphism. An alternative explanation could be that the most sexually dimorphic characters were the same as those which varied most with age, indicating a neotenic pattern of development for male *X. l. laevis*. Whilst it is possible that one factor exacerbated the other, determining which of these interpretations had the greater impact on results would have required that the osteological sample had contained older male specimens, absent from the Californian field-sample.

Given the weak correlation between size and age in females, a comparison of osteological variation between these two criteria was made. Results returned by multivariate analyses for each study were highly congruent, albeit the relative degree of variation for each of the identified characters differed between studies. Overall however, no significant difference was detected between the effects of these two criteria on osteology.

Owing to congruence between results from each study, differences in the rate of correct classification by DFA were also compared with a view to identifying which of the three criteria, sex, age or size, accounted for the greatest degree of osteological variation. The rate of correct classification was highest for groups classified by sex, but as explained above, the focus here may have been on differences in age between samples, rather than on sexual dimorphism *per se*. Rates of correct classification in each of the remaining studies were low. Non-sequential ordering of age- and size-categories indicated a weak correlation between overall variation and each of these criteria. It appears that whilst a number of characters were identified as varying significantly with age and size, they do not necessarily co-vary within the same specimen, resulting in staggered clines of character-variation across the sample.

CONCLUSION

Although it has not been possible to draw distinctions between the variable effects of sex, age and size on osteology, results have identified the most common manifestations of osteological variation in *X. l. laevis*. It is likely that other variables, both intrinsic and extrinsic, may profoundly affect osteology, and to seek a close correlation between any single variable and overall osteological variation may be over-simplistic and unrealistic. Nevertheless, it has also been possible to identify characters that demonstrate isometric growth and which can therefore be used to characterize the taxon unambiguously.

The present study of osteology highlights a need for caution in future inter-specific comparative studies that characterize taxa according to their osteological attributes. Results cannot be applied directly to studies that utilize other *Xenopus* taxa, since variation demonstrated by characters in *X. l. laevis* may not correspond directly to the form of variation taken by these characters in other taxa (e.g. see chapter 6). Indeed, a wholly different suite of characters may represent those demonstrating greatest variation in other species. Evaluations of the taxonomic significance of *X. l. laevis* characters in this study serve merely as a source of reference for future studies that employ *X. l. laevis* as a model taxon for osteological study, and as a reminder of the potential significance of intra-specific osteological variation within the context of inter-specific comparative studies.

Appendix 1- character list. All characters except for those that are asterisked are categorical.

Skull
1 maximum width of skull including tympanic annulus
1b maximum width occipitopetrosal
2 maximum length of skull to base of foramen magnum

Nasal
3 width at point of association with frontoparietal
4 maximum width nasal
5 length nasal

Septomaxillary
10 distance between lateral edge of nasal and lateral edge of septomaxillary (perpendicular to main body axis)
11 length of gap between maxillary arch and lateral edge of septomaxillary
12 gap between anterior margin of nasal and anterior margin of septomaxillary (along main body axis)

Rostral calcification
14 alary process
15 nasal floating cartilage (Element A of Smirnov, 1994)
16 nasal flanking cartilage
16b planum antorbitale

Frontoparietal
17a length frontoparietal
17b frontoparietal to occipitopetrosal base
19 width at level of association with occipitopetrosal
20a length anterior from level of association with occipitopetrosal to tip of frontoparietal
21 length posterior to foramen magnum base
22 length from tip of frotoparietal to pineal foramen
23 length from anterolateral alae level to pineal foramen
24 length posterior from pineal foramen
*25 parasagittal crests close parallel, moderately close and parallel, medium width diverging, wide diverging- 0,1,2,3,4.

Occipitopetrosal
29 width of foramen magnum in dorsal view
30 depth of foramen magnum in dorsal view
30b width/depth foramen magnum
31 distance between medial margin at base of knobbles

Vomer
*33a azygous, paired, fractured- 0,1,2
*33b fused unfused 1/0
34 maximum width vomer

Sphenethmoid
36 anterior width of sphenethmoid
37 length of columella from pars palatina to level of anterolateral alae
*38 orbitonasal foramen absent, present. 0/1
39 length of optic foramen
40 distance between medial margins of rtbs

Tympanosquamosal
41 width of anterior end of columella in dorsal view
42 length between medial margins of columella
43 length of zygomatic process
44 length of tympanic annulus
47 length of anterior ramus of zygomatic process
*48 curvature of tympanic annulus/ zygomatic process junction - none, gradual, deep, very deep 0/1/2/3

Maxillary arch
*49 square ended/ tapered ended max 0/1
50 length of premaxillary
51 length from anterior dental ridge of maxillary to posterolateral corner
52 length from anterolateral dental ridge to end of dental ridge
53a distance between tooth row on anterior margin and level of post margins
53b distance between anterior margin (p facialis level) and level of post margins
54 number of teeth on premaxillary
55 number of teeth of maxillary
56 width of facial flange at maxillary/ premaxillary junction
57 width of pars palatina at junction between premaxillaries
*58 maxillary extends beyond anterior ramus of pterygoid/ doesn't 1/0
*59 overlap/ no overlap between maxillary and premaxillary 1/0
60 difference in level between medial and lat edges of premaxillary
*61 degree of overlap between post margins 0/1/2

Pterygoid
62 distance between medial margins of otic plates
63 distance between medial margins at base of main pterygoid arm
65 half width of skull at point of association with maxillary
66 width of orbit at point of overlap between pterygoid and maxillary arch
67 length of anterior ramus- to lateral edge

Degree of synostosis
*68 frontoparietal and nasal 0/1
*69 frontoparietal and occipitopetrosal 0/1
*70 sphenethmoid and occipitopetrosal 0/1

Vertebral Column

71 width of atlas in dorsal view
71b length of prehallux
72 depth of atlas in dorsal view
73 length of vertebral column
74 width of 1st transverse process
75 2nd transverse process
76 3rd transverse process
77 length of sacral diapophysis
78 length of urostyle
*79 degree of imbricateness 0/1/2.

Hindlimb

82 femur length

83 fibio-tibula length
84 fibiale-tibulare length
85 length 2nd metatarsal

Forelimb

86 humerus length
87 radio-ulnare length
88 2nd metacarpal length

Pelvic girdle

89 length iliac shaft
90 length ischium in dorsal view
91 width acetabular- anterior margin
92 width acetabular- posterior margin

Pectoral Girdle

*93 cleft, scarred, unscarred scapula- 2/1/0
94 Clavicle medial width
95 Distance between anterior tip of clavicle and anterior tip of coracoid
96 length along posterior margin of scapula (distance between lateral corner and tip of pars glenoidialis)

Appendix 2

The following table lists characters whose coefficients were either negative or moderate-to-low in magnitude for PCA1. Each character, standardized against s-v length, was regressed against and correlated with s-v length to assess its relationship with body length. Sexes were analyzed separately so as not to mask effects of sexual dimorphism. R^2 gives a value for the explanatory power of the regression model, and correlation value of 0 indicates isometric growth of that character. Asterisks refer to t-test significance levels for sexual dimorphism (see Table 3-1).

Character number	Character	PCA component score	Sex	P, F, r^2 (male, female)	Correlation
40	Distance between rtbs	0.193492	M	0.737,0.12,1.3%	0.162
			F	0.286,1.2,5.4%	0.225
43	Zygomatic ramus length	0.549862	M	0.94,0.01,0.1%	-0.072
**			F	0.001,15.21,43.2%	0.081
54**	Number of pre-maxillary teeth	0.045385	M	0.002,18.99,67.8%	-0.82
***			F	0.000,51.85,71.2%	-0.84
55	Number of maxillary teeth	0.44707	M	0.36,0.92,9.3%	0.785
			F	0.443,0.61,2.8%	0.925
87	Radio-ulnare length	0.574097	M	0.834,0.5,0.5%	0.072
			F	0.668,0.19,0.9%	-0.095
98	Angle between coracoid and zonal plane	-0.33336	M	0.374,0.88,8.9%	-0.934
***			F	0.000,87.16,80.6%	-0.869

Table 3-11

Univariate statistical results listed in this table showed characters to either vary independently or decrease in relative size with s-v length. Distance between rtbs, maxillary tooth count and radio-ulnare length all varied independently of s-v length, whilst the relative number of pre-maxillary teeth decreased. In males, the zygomatic ramus was highly variable, showing a relative decrease in length, whilst in females, length was much more strongly related, and positively correlated with s-v length. Abbreviations: rtbs: retractor bulbi muscle scars

Appendix 3

Recipes

Formal-Nitric decalcifying fluid- Nitric acid 1ml, Formalin, 0.5 ml, distilled water, 100ml.
Harris' Haematoxylin- HH BDH 34124 5g, Ethanol 100g, Ammonium alum 50g, Distilled water 900ml. Dissolve alum in water by warming. Add H. Bring to boil. Take off heat. Add 0.5g Sodium iodate and stir. Cool rapidly by immersion in water. Filter and add 40ml Glacial acetic acid.
Scott's Tap water- Potassium bicarbonate 2g, Magnesium sulphate 20g, Distilled water 1000ml. (see Mahoney, 1973).

BIBLIOGRAPHY

Baez, A.M. (1981). Redescription and relationships of *Saltenia ibanezi*, a late Cretaceous Pipid from Northewest Argentina. *Ameghiniana* **18(3-4)**:127-154.

Baez, A.M. and Trueb, L (1997). Redescription of the Paleogene *Shelania pascuali* from Patagonia and its bearing on the relationships of fossil and recent pipoid frogs. *Scientific papers, Natural History Museum of the University of Kansas* **4**:1-41.

Butcher, G.A. (1980). Multivariate analysis of hybridizing Cyprinidae from the Kanansaskis reservoirs, Alberta. *Canadian Journal of Zoology* **58**:1664-1673.

Caetano, M.H., Castanet, J. and Francillon, H. (1985). Determination de l'age de *Triturus marmoratus marmoratus* (Latreille, 1800) du Parc National de Peneda Geres (Portugal) par squelettochronologie. *Amphibia-Reptila* **6**:117-132

Cannatella, D.C. and Trueb, L. (1988). Evolution of pipoid frogs: intergeneric relationships of the aquatic frog family Pipidae (Anura). *Zoological Journal of the Linnean Society* **94**:1-38.

Castanet, D.C., Francillon-Vieillot, H., Mieunier, C. and Riqules, A. 1993. "Bone and individual ageing." In Bone Growth – B. B.K.Hall, Ed. CRC Press, Boca Raton, Vol. 7, pp245-283.

Castanet, J. and Smirina, E.M. (1990). Introduction to the skeletochronological method in amphibians and reptiles. *Annals of Science, Nature Zoology, Paris* **11**:191-196.

Castanet, J and Baez, M. (1991). Adaptation to evoution in Gallotia lizards from the Canary Islands: Age, growth, maturity and longevity. *Amphibia-Reptilia* **12**:81-102.

Cherry, M.I., and Francillon-Vieillot, H. (1992). Body size, age and reproduction in the leopard frog, *Bufo pardalis*. *Journal of Zoology, London* **228**:41-50.

Darwin, C. 1871. Descent of man and selection in relation to sex. New York: D. Appleton Company.

Deuchar, E. 1975. *Xenopus*, The African Clawed Frog 1st ed. London: John Wiley and Sons, Ltd.

Duellman, W.E. and Trueb, L. 1986. Biology of Amphibians 1st ed. New York, St. Louis, San Francisco: McGraw-Hill Book company.

Emerson, S.B. (1979). The ilio-sacral articulation in frogs: form and function. *Biological Journal of the Linnean Society* **11**:153-168.

Emerson, S.B. (1982). Frog postcranial morphology: identification of a functional complex. *Copeia* **1982**:603-613.

Estes, R. and Reig, O.A. 1973. "The early fossil record of frogs. A review of the evidence." Pp. 11-63 in Evolutionary Biology of the Anurans: Contemporary Research on Major Problems, J. L. Vial, ed. Columbia: University of Missouri Press.

Fabrezi, M (1993). The anuran tarsus. *Alytes* **11(2)**:47-63.

Fabrezi, M (1996). The carpal elements of anurans. *Herpetologica* **52(2)**:188-204.

Fabrezi, M (2001). A survey of prepollex and prehallux variation in anuran limbs. *Zoological Journal of the Linnean Society* **131**:227-248.

Francillon-Vieillot, H., Arntzen, J.W. and Geraudie, J. (1990). Age, growth and longevity of sympatric *Triturus cristatus*, *T. marmoratus* and their hybrids (Amphibia, Urodela): A skeletochronological comparison. *Journal of Herpetology* **24 (1)**:13-22.

Griffiths, I (1954). On the nature of the frontoparietal in Amphibia, Salientia. *Proceedings of the Zoological Society of London* **123**:781-792.

Harper, D.G.C. (1994). Some comments on the repeatability of measurements. *Ringing and migration* **15**:84-90.

Halliday, T.R. and Verrell, P.A. (1988). Body size and age in amphibians and reptiles. *Journal of Herpetology* **22, 3**:253-265.

Hemelaar, A. (1985). An improved method to estimate the number of year rings resorbed in phalanges of *Bufo bufo* (*L.*) and its application to populations from different latitudes and altitudes. *Amphibia-Reptilia* **6**:323-341.

Jurgens, J.F. (1971). The morphology of the nasal region of Amphibia and its bearing on the phyogeny of the group. *Annals of the University of Stellenbosch* **46A(2)**:3-136.

Klingenberg, C.P., Spence, J.R. and Mirth, C.K. (2000). Introgressive hybridization between two species of waterstriders (Hemiptera: Gerridae: *Limnoporus*): geographical structure and temporal change of a hybrid zone. *Journal of evolutionary biology* **13**:756-765.

Kobel, H.R., Loumont, C. and Tinsley, R.C. 1996. "The extant species." Pp. 9-33 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. 1st ed. Oxford: Clarendon Press.

Leclair Jr., R. and Castanet, J. (1987). A skeletochronological assessment of age and growth in the frog *Rana pipiens* Schreber (Amphibia, Anura) from southwest Quebec. *Copeia* **1987**:361-369.

Mahoney, R. 1973. Laboratory techniques in Zoology. London, Butterworths Press.

McCoid, M.J. and Fritts, T.H. (1989). Growth and fat body cycles in feral populations of the African Clawed frog, *Xenopus laevis* (Pipidae), in California with comments on reproduction. *The Southwestern Naturalist* **34(4)**:499-505.

Measey, G.J. (1997). Ecology of feral *Xenopus laevis*. Ph.D dissertation, University of Bristol.

Measey, G.J. and Channing, A. (2001). When is *Xenopus laevis* not *Xenopus laevis*? A Phylogeographic approach to the genus *Xenopus* in Southern Africa. *(unpublished).*

Measey, G.J. and Wilkinson, M. (1998). Lines of arrested growth in the caecilian, *Typhlonectes natans* (Amphibia: Gymnophonia). *Amphibia-Reptilia* **19**:91-95.

Measey, G.J. and Tinsley, R.C. (1998). Feral *Xenopus* in South Wales. *Herpetological Journal* **8 (1)**:23-27.

Miaud, C., Andreone, F., Riberon, A., De Michelis, S., Clima, V., Castanet, J., Francillon-Vieillot, H. and Guyetant, R. (2001). Variations in age, size at maturity and gestation duration among two

neighbouring populations of the Alpine salamander (*Salamandra lanzai*). *Journal of Zoology, London* **254**:251-260.

Miaud, C., and Guyatant, (1998) Elasticity and selection on life-history traits in a complex life-cycle organism, the common frog *Rana temporaria* (Amphibia: Anura). *Bulletin de la Societe Zoologique de France* **123(4):**325-344

Miaud, C., Guyetant, R. and Elmberg, J (1999). Variations in life history traits in the common frog *Rana temporaria* (Amphibia: Anura): a literature review and new data from the French Alps. *Journal of Zoology, London* **249**:61-73.

Palmer, M. (1960). Expanded ilio-sacral joint in the toad *Xenopus laevis* (Pipidae: Anura). *Nature* **187**:797-780.

Parker, H.W. (1934). Reptiles and amphibians collected by the Lake Rudolf Rift Valley expedition, 1934. *Annals and Magazine of Natural History* **18**:594-609.

Paterson, N.F. (1939). The head of *Xenopus laevis*. *Quarterly Journal of Microscopical Science* **81**:161-234.

Reumer, W.F. (1985). Some aspects of the cranial osteology and phylogeny of *Xenopus* (Anura; Pipidae). *Revue Suisse Zoologie* **92** (4):969-980.

Ryan, B.F., Joiner, B.L., Ryan, T.A. (1985). *MINITAB Handbook* (second edition). Duxbury Press: New York, NY.

Ryser, J. (1988). Determination of growth and maturation in the common frog, *Rana temporaria*, by skeletochronology. *Journal of Zoology, London* **216**:673-685.

Sassoon, D. and Kelley, D.B. (1986). The sexually dimorphic larynx of *Xenopus laevis*: development and androgen regulation. *The American Journal of Anatomy* **177**:457-472.

Smirnov, S.V. (1994). Postmaturation skull development in *Xenopus laevis* (Anura, Pipidae): late-appearing bones and their bearing on the pipid ancestral morphology. *Russian Journal of Herpetology* **1**, **1**:21-29.

Tinsley, R.C. and McCoid, M.J. 1996. "Feral populations of *Xenopus* outside Africa." Pp. 81-120 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Thorpe, R.S. (1976). Biometric analysis of geographic variation and racial affinities. *Biological Review* **51**:407-452.

Thorpe, R.S. (1983). A biometric study of the effects of growth on the analysis of geographic variation: tooth number in Green Geckos (Reptilia, Phelsuma). *Journal of Zoology, London* **201**: 3-26.

Tinsley, R.C. and Tocque, K. (1995). The population dynamics of a desert anuran, *Scaphiopus couchii*. *Australian Journal of Ecology* **20**:376-384.

Trueb, L. and Hanken, J. (1992). Skeletal development in *Xenopus laevis* (Anura:Pipidae). *Journal of Morphology* **214**:1-41.

Trueb, L. and Cannatella, D.C. (1986). Systematic, morphology and phylogeny of the genus *Pipa* (Anura: Pipidae). *Herpetologica* **42**:412-449.

Willig, M.R., Owen, R.D. and Colbert, R.L. (1986). Assessment of morphometric variation in natural populations: the inadequacy of the univariate approach. *Systematic Zoology* **35(2)**:195-203.

Zar, J. H. 1984. Biostatistical analysis. 4th Edition. Prentice Hall International inc., London

CHAPTER 4 : OSTEOLOGICAL EVIDENCE FOR THE HYBRID ORIGIN OF OCTOPLOID SPECIES, *X. VESTITUS* AND *X. WITTEI* FROM PREDECESSORS OF TETRAPLOIDS *X. FRASERI* AND *X. L. VICTORIANUS* IN THE CENTRAL AFRICAN HIGHLANDS

INTRODUCTION

The central African highlands are characterized by high species diversity and endemism, not only for *Xenopus* (see intro; Tinsley, 1981a) but also for a range of other indicator groups including amphibians and reptiles (Howell, 1993), butterflies (Carcasson 1964; De Jong and Congdon, 1993), birds (Moreau, 1962; Fjeldsa and Lovett, 1997; Fjeldsa, Ehrlich, Lambin and Pins, 1997) and land mollusks (Tattersfield, 1998). This distribution pattern is consistent with the area having acted as an ice-age refugium during successive cool, arid periods of the Pleistocene (Hamilton, 1976; Bonnefille, Riollet, Buchet, Icole, Lafont and Arnold, 1995; Fjeldsa and Lovett, 1997; Jolly, Taylor, Marchant, Hamilton, Bonnefille, Buchet and Riollet, 1997; Marchant, Taylor and Hamilton, 1997).

Seven *Xenopus* taxa are documented as living, or having recently lived in the vicinity of these highlands, 4 of which are endemic. Of these, *X. wittei, X. vestitus* and *X. l. bunyoniensis* occur in eastern Democratic Republic of the Congo (DRC), northern Rwanda and southwestern Uganda, the fourth, *X. ruwenzoriensis*, in the Semliki valley, north of the Ruwenzories in Uganda. The latter two areas are specifically characterized by species richness and endemism peaks (DeKlerk, Crowe, Fjeldsa and Burgess, 2002). Typically for endemics, their distribution ranges are relatively narrow compared with those of non-endemic *X. fraseri, X. pygmaeus* and *X. l. victorianus*, each having wider distributions on either side of the western Rift Mountains. This study will focus on the possibility that octoploid *X. wittei* and *X. vestitus* are each the allopolyploid hybrid product of tetraploid predecessors of *X. fraseri* and *X. l. victorianus* whose ranges are likely to have repeatedly come into contact in these highlands during the Pleistocene.

Topography of the Central African Highlands

The interlacustrine highlands that overlap borders between Rwanda, Uganda and DRC were formed by uplifting of Precambrian crystalline rock (since the middle Tertiary, 25MYA) and more recently (late Tertiary and Quaternary) by faulting and volcanism within the Western Rift system (Hamilton, 1982; Lovett, 1993). Although volcanic activity in the Western Rift occurred mainly during the Miocene, it is ongoing and has caused damming of some valleys, the formation of a series of lakes and the reversal of major drainage courses (Tinsley, 1973). Today, this highland complex forms a significant watershed, narrowly separating drainage to Lake Albert and the Albert Nile in the

north from the savannas of east Africa and low-lying forests in the Congo basin to the west, whilst acting as a physical boundary separating many taxa living on either side of the Western Rift. During climatically favourable periods of the Pleistocene, conditions of increased humidity and temperature spread through the continent. This enabled expansion of forest ranges, and in so doing, provided a migration corridor for taxa living in habitats in eastern DRC and the Congo basin between lakes Kivu and Albert (Hamilton, 1982).

Lake Kivu lies within the topographically complex area that borders Congo and Rwanda and was formed by the damming of the Rugika valley by lava flows from the Virunga volcano complex (Visser, 1962; Tinsley, 1975; Tinsley, 1981b). Whilst Kivu now drains to Tanganyika in the south, highlands to the northeast of the lake form a watershed separating the Kivu, Edward and Albert drainage systems (Tinsley, 1975) (see Figure 4-1, Figure 4-2). The interlacustrine area between Lakes Kivu and Edward form the focus of the distribution of the four taxa of interest in the current study (Figure 4-2).

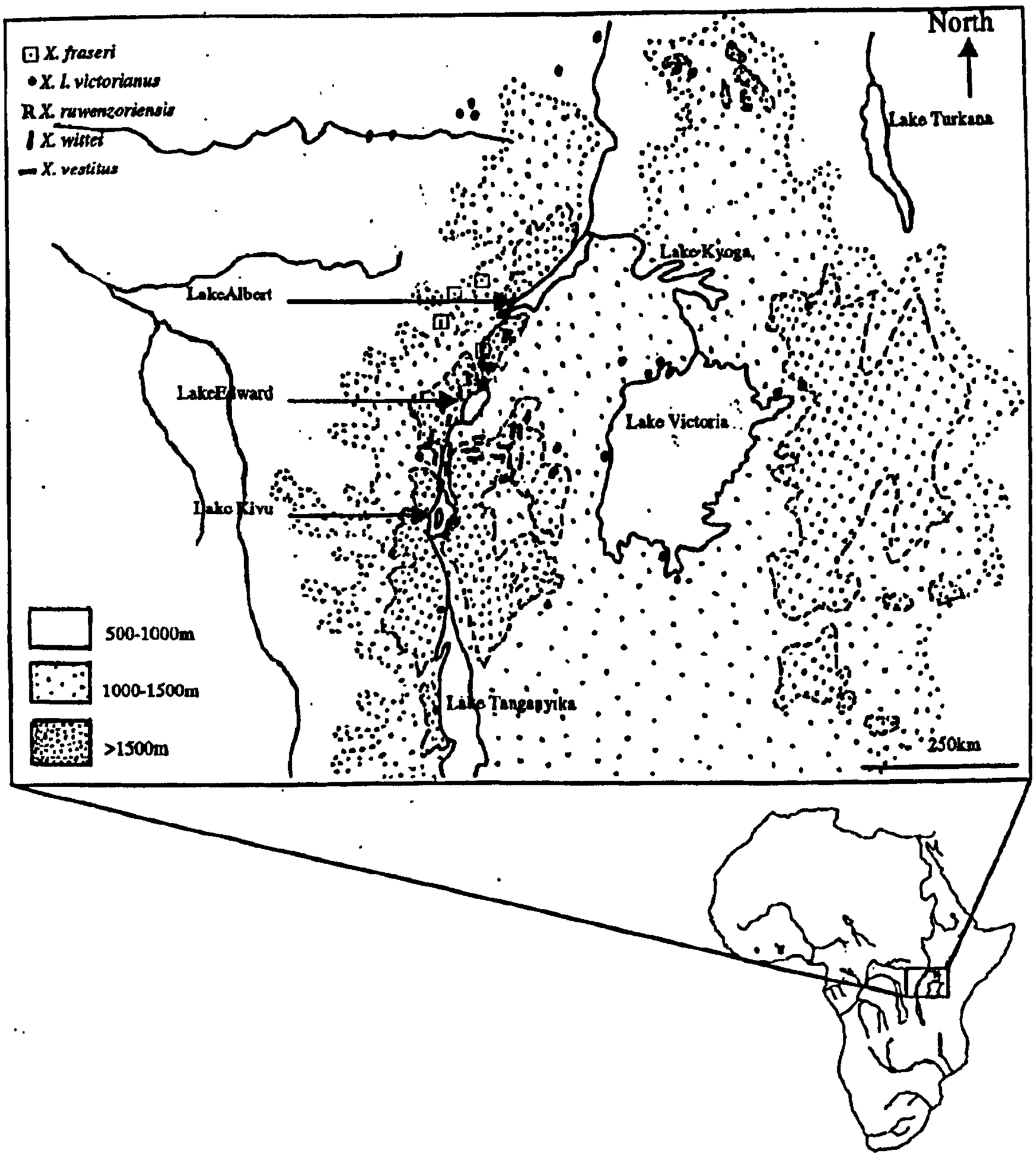


Figure 4-1

Map of central Africa (modified from Tinsley and Kobel, 1996).

Map of Central Africa showing
distribution of highland lakes

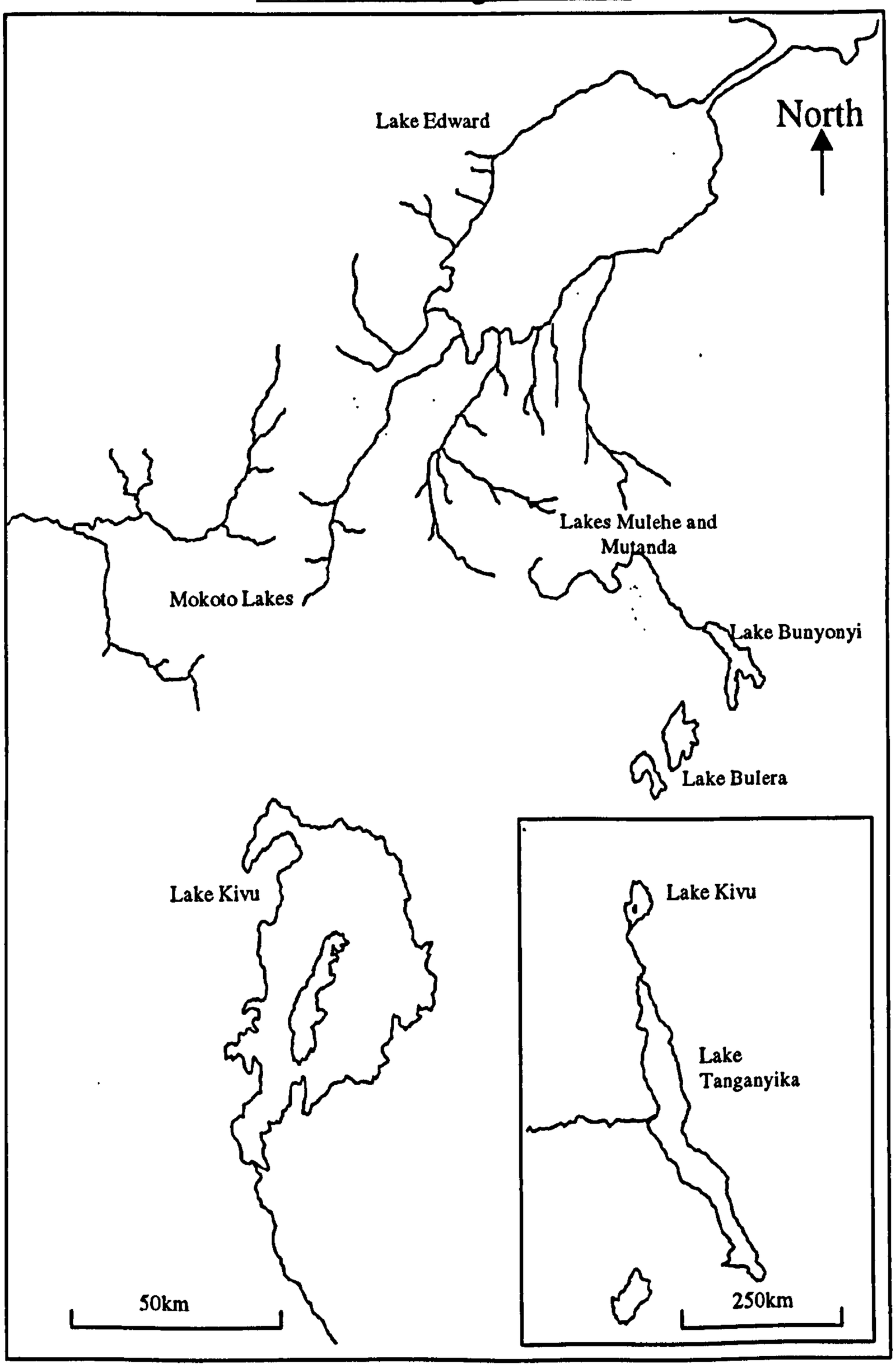


Figure 4-2

Map of interlacustrine region to the north of Lake Kivu (modified from Tinsley and Kobel, 1996).

Species distributions

Details are presented for each of the taxa whose combined distribution ranges form a focal point for diversity in the highlands of the Western Rift.

Xenopus laevis victorianus (tetraploid) Ahl, 1924 has long been known from areas to the east of the Kigezi highland complex in southwestern Uganda. Its distribution is now known to stretch from the type locality near lake Victoria westwards as far as Burundi, Rwanda, eastern and northeastern DRC and Uganda, eastwards into northern parts of Tanzania and southwestern Kenya, the northern margin of the distribution range extending as far as southern Sudan. Although *X. l. victorianus* occurs primarily between 1000 and 1800m (Tinsley, Loumont and Kobel, 1996), published accounts of this species occurring in wooded grassland at higher altitudes are well substantiated.

Xenopus fraseri (tetraploid) Boulenger, 1905 was described from West Africa. Although no specific locality details are provided in the original account, it is thought that type material was collected in Bioko (Fernando Póo) or Nigeria (Tinsley *et al.*, 1996). Whilst most investigations have been based on tetraploid material from Sangmelima, Cameroon, at present, at least six *X. fraseri*-like taxa of varying ploidy levels are recognized (tetra-, octo- and dodecaploid; see thesis introduction). Ecological details regarding the distribution ranges of these taxa, however are lacking. Although the *X. fraseri*-like species used in the present study is known to be tetraploid (Tinsley, pers. com), its origin in eastern DRC is far from the type locality, and it could therefore represent an as yet un-described form (Tinsley *et al.*, 1996). Its geographic position, relative to the central highlands, makes it one of two possible *X. fraseri*-like taxa to have been involved in the hybridization events that gave rise to *X. vestitus* and *X. wittei*. Another candidate is *X. pygmaeus*, also distributed in lowland forests of eastern DRC. The former taxon will be referred to hereafter as *X. fraseri aff.*, since its relationship to the western *X. fraseri* form is unknown. (Jackson, Tinsley and Kigoolo, 1998).

Xenopus vestitus (octoploid) Laurent, 1972 was described almost simultaneously in two independent accounts: by Laurent, as *X. vestitus*, and by Tinsley (1973), as *X. kigeziensis*. Whilst Laurent based his description on museum specimens collected in the former Albert National Park (Parc National des Virungas), the description by Tinsley was based on live specimens collected from southwestern Kigezi. These were later synonymized (Tinsley, 1975). A complete type-series of 77 specimens was collected by deWitte in 1945-55 and a single specimen by Donis in 1957, from rivers near Rutshuru and from Kinyantuku, Kivu. *Xenopus vestitus* has been collected from sites in eastern DRC, southwestern Uganda and northern Rwanda (Tinsley, 1975).

Xenopus wittei (octoploid) Tinsley, Kobel and Fischberg, 1979 was first recognized in the paratype series for *X. l. bunyoniensis* collected in 1925, but was not formally described for another fifty years (Kobel, Loumont and Tinsley, 1996). The *X. wittei* holotype was collected by Tinsley,

Kobel and Fischberg (1979) from Chelima forest, Kigezi at an altitude of 2200m. The taxon has been collected from the same regions as those listed above for *X. vestitus*.

Xenopus l. bunyoniensis (ploidy unknown) Loveridge, 1932 was widespread in the lakes of southwestern Uganda, and is documented as having occurred in lakes Bunyonyi, Mutanda, Mulehe, Bulera, Chahafi and Mamvu and also in Echuya and Kajonza forests (Tinsley, 1973, 1975; Tinsley *et al.*, 1979). Each of these localities is currently occupied either by *X. vestitus, X. wittei* or *X. l. victorianus*, with *X l. bunyoniensis* having not been recorded there since the early 1940's, with the exception of a single specimen collected in 1975 (Tinsley, 1981b). Replacement of *X. l. bunyoniensis* in these lakes is postulated to have occurred during the second half of the last century (Tinsley, 1981b).

Evidence from museum records for a recent change in species distribution

Recent changes in the distribution of these species in the complex of central highland lakes have been inferred from the museum record (Tinsey, 1973). There is evidence for a recent migration of *X. vestitus* to the Kigezi lakes from lakes to the south and west, based on historic data from material collected by Laurent in Kivu province (Tinsley, 1973). *Xenopus l. victorianus* was known to occupy lakes to the northeast, east and south prior to its migration to this lake complex, and the lakes themselves were previously occupied by *X. l. bunyoniensis*, now possibly extinct (Tinsley, 1981b). Like *X. vestitus*, *X. wittei* has probably been widespread for a longer period than is represented by museum collections. Its absence from early records may be explained by a previous tradition of collecting from low-lying areas (Tinsley, 1981b). This explanation is consistent with *X. wittei* having a more extensive distribution at higher elevations (Alt. 1600-2600m) whilst the range of *X. vestitus* extends to lower altitudes (Alt. 1200-2000m) (Tinsley, 1975; Tinsley *et al.*, 1979). First recognized in museum collections from Lake Bunyonyi (Tinsley, 1973, 1975), *X. wittei* was later found to be widespread throughout the region, living in habitats ranging from papyrus at Lake Bunyonyi and Mokoto to bamboo at Mount Tshiaberimu and lowland rainforest in the Impenetrable forest, southwestern Uganda (Tinsley *et al.*, 1979).

Sympatry in the Central African Highlands

The current pattern of species distribution broadly reflects constraints imposed by climatic fluctuation during the Pleistocene. Whilst it is recognized that zones of overlap between species occupying broad ranges are generally narrow (Tinsley, 1981b), the ranges of species with narrower distributions, living in areas of high diversity, are less exclusive. Instability on a local scale, as is indicated by recent changes in the distribution of taxa living in these highlands, and the heterogeneity of habitats that lie within close proximity to one another (Kobel, Du Pasquier and Tinsley, 1981), may result occasionally in spatial overlap between species ranges. However, it is likely that sympatric sites

represent labile zones of competition rather than the ecological limits of species ranges (Tinsley, 1981b).

Several cases of sympatry in these highlands have been documented during fieldwork. Many more are based on museum collections. Since it is possible that species collected from sites in close proximity would have been mixed within the same collection sample, care must be exercised if inference is to be drawn from this latter source (Tinsley, 1981b; Tinsley *et al.*, 1979). Sympatry has been documented for *X. wittei* and *X. l. victorianus* in Mukaka, Rwanda at an altitude of 2200m (Tinsley *et al.*, 1996), for *X. vestitus* and *X. l. victorianus* at a number of sites, and also for *X. wittei* and *X. vestitus* (Tinsley, 1975; Tinsley *et al.*, 1979). *Xenopus wittei*, *X. vestitus* and *X. l. victorianus* have been found in breeding condition together at a site in Pinga, eastern Congo, at altitudes ranging from 1200-2000m (Tinsley, 1975; Tinsley *et al.*, 1979; Tinsley and Kobel, 1996), and at a second site, but locality details pertaining to this record are dubious (Tinsley *et al.*, 1979). Another endemic, *X. l. bunyoniensis* Loveridge, 1932, was found living sympatrically with *X. wittei* in lake Bunyonyi in 1975 (Tinsley, 1981b). Despite their ecological differences, Laurent (1972) also reported having found *X. l. victorianus* and *X. fraseri* living sympatrically in a small number of water bodies in eastern DRC, a situation likely to have been more common during past periods of greater forest expansion (Tinsley, 1996).

Among the processes driven by conditions of sympatry would have been a breakdown in positive-assortive mating, leading in turn to an increased incidence of miss-pairings (Gollmann, 1991), and in some cases to hybridization. The probability of hybridization will have been accentuated if, as documented (see above), species living sympatrically were to share the same breeding season.

Phylogenetic relationships

The discovery that both *X. wittei* and *X. vestitus* have an octoploid karyological constitution and are allopolyploid (Thiebaud and Fischberg, 1977; Tymowska, 1977; Tymowska and Fischberg, 1980) came at a time when more was being understood about the reticulate mode of evolution in the genus and its biogeographical significance. That the ranges of more widely distributed species will have been restricted to refugia during periods of increased aridity, and that hybridization between tetraploid species has been shown in breeding experiments to lead to the production of allopolyploid hybrids (Kobel *et al.*, 1986) introduces the possibility that octoploids currently occupying restricted range distributions in these highlands could be the hybrid product of tetraploid species whose ranges either extend into or lie adjacent to those of octoploid species. A number of studies have attempted to determine the relative phylogenetic position of these species, yet precise relations remain ambiguous (Tinsley and Jackson, 1998a,b).

Experimental hybridization between the two octoploids revealed that half their chromosomes form bivalents when hybridized (Kobel and Muller, 1977) suggesting that the species pair share a common ancestor. Comparative karyology has indicated that a *X. laevis* sub-species probably represents one of the parent species of *X. vestitus* (Tymowska, 1977). Further evidence allying *X. vestitus* with *X. laevis* is provided by results of comparative studies of mtDNA (Carr, Brothers and Wilson, 1987), parasitology (Tinsley, 1996) and globin peptides (Burki and Fischberg, 1985). Studies comparing mDNA (Kobel, Barandun and Thiebaud, 1998), mitochondrial rDNA and albumin protein (Graf and Fischberg, 1986) found a strong association between *X. fraseri aff.* and *X. wittei*. A complex pattern of shared monogenean parasites (*Protopolystoma* species- characterized by strict host specificity) was, until recently, believed to accord with the presumed genetic evolution of octoploid hosts (Tinsley, 1996) in implying that *X. wittei* was the product of hybridization between *X. l. victorianus* and *X. fraseri aff.* However, preliminary results from molecular analyses of these parasites have cast doubt on the strength of this evidence (Tinsley, pers. com.). The only evidence linking *X. vestitus* with *X. fraseri aff.* comes from a comparative study of globin peptides (Burki and Fischberg, 1985).

Whilst assimilated evidence indicates that both octoploids share common *X. laevis* and *X. fraseri aff.* ancestors, evidence linking *X. vestitus* with *X. fraseri aff.* is limited to results from a single study and contrasts with those from a range of more recent studies, which suggest that *X. vestitus* occupies a relatively isolated position with respect to *X. fraseri aff.*, *X. l. victorianus* and *X. wittei* (Graf and Fischberg, 1986; Graf, 1996; Kobel *et al.*, 1998; Tinsley and Jackson, 1998a).

Osteology

That comparative osteology has been neglected as a method for determination of the systematics of species within the genus is somewhat surprising given its obvious potential, as demonstrated by the only published documentation of its application in this respect; the genus was split into three groups by Reumer (1985), albeit based on a conservative number of specimens. Amongst other *Xenopus* taxa, these included a single specimen of *X. fraseri aff.*, *X. vestitus* and *X. wittei* and two specimens of *X. l. victorianus*. Although only cranial characters were considered in the inter-species comparison, taxonomic groupings correspond to a certain degree with results from recent phylogenetic studies (Graf, 1996; Kobel *et al.*, 1998). Reumer associated *X. wittei* with *X. fraseri aff.* and although *X. vestitus* was not incorporated into any of the groups, he acknowledged that the species shares a number of affinities with *X. l. victorianus*.

Whilst the current political situation in these highlands makes the possibility of collecting material logistically difficult, museum collections from this locality are extensive and provide the potential to conduct a study of comparative osteology using wild-caught material. An approach such

as this maximizes the potential of museum collections whilst negating the need for lab-raised or wild-caught live material.

The choice of *X. l. victorianus* and *X. fraseri aff.* for use in this study to determine relations for octoploids *X. wittei* and *X. vestitus* is based not only on current distribution data, but also on prior knowledge of affinities within the genus. This is founded on published phylogenetic accounts (Graf, 1996; Kobel *et al.*, 1998), on natural groupings indicated by osteological affinities within the genus (pers. obs.) and on current geographical distribution. The only tetraploid taxa currently known to occupy areas surrounding these highlands are *X. pygmaeus* Loumont, 1986, *X. fraseri aff.* and *X. l. victorianus*. No known museum collections of the former taxon exist, precluding their inclusion in the current study. Although *X. muelleri* occurs in lowland areas adjacent to those occupied by *X. l. victorianus*, *X. muelleri* is absent from the vicinity of these highlands and fundamental differences exist between *X. vestitus*, *X. wittei* and taxa in the *X. muelleri* group, as indicated by a range of molecular, biochemical and comparative osteological studies (Graf, 1996; pers. obs.). Whilst it is possible that other tetraploid taxa exist or have existed in the surrounding area, currently there is no obvious taxonomic or geographical justification for the inclusion of tetraploid taxa other than those already implicated in the hybrid origin of this octoploid pair.

AIMS

The present study aims to apply methods of comparative osteology, using museum and university collections, to assess evidence of inter-relatedness between these four taxa. Phenetic similarities will be determined using osteometric data and evaluated for any implication of the involvement of tetraploids in the allopolyploid event that gave rise to octoploids *X. wittei* and *X. vestitus*. Visual examination of specimens and statistical analysis of osteometric data will be used to identify the form of inter-taxon variation.

Descriptions of cranial and post-cranial osteology will be presented for the first time for each taxon, with an emphasis placed on inter-specific variation. These will form part of a broader series presented in this thesis and will serve as a key to the identification of species examined during this study, since identification by external morphology alone can be problematic.

MATERIALS AND METHODS

Materials

Sixty-eight sexually mature adult specimens were cleared and single-stained for osteological examination. These included 17 *X. fraseri aff.* (9 females, 8 males), 15 *X. l. victorianus* (8 females, 7 males), 17 *X. wittei* (9 females, 8 males) and 19 *X. vestitus* (14 females, 5 males, 3 sex unrecorded).

Locality details

Whilst most of this material came from R. C. Tinsley's private collection, additional specimens came from a collection donated by the Salta Museum of Natural History, Argentina. *Xenopus fraseri* came from Irangi, Lomami, Bushoho, Hombo, Bikoro (DRC); *X. wittei* from Rwasenkoko, Chelima, Echuya, Bunyonyi (S.W. Uganda); *X. l. victorianus* from Chahafi, Kipoyo (Kivu), Nyamata (Kigali); *X. vestitus* from Mulehe, Mutanda (S.W. Uganda).

Methods

Snout-vent length was recorded for each specimen prior to clearing and single-staining, following methods outlined in chapter 2. A total of 74 characters (60 continuous and 14 categorical) were measured at a magnification of x10 using a stereo binocular microscope (Leica) fitted with an ocular micrometer, using fibre-optic illumination. Characters are listed by number in Appendix 1, chapter 3.

Statistics

Data were tested for repeatability following the methods of Harper (1994) (see chapter 3). Mean snout-vent length was determined for both sexes of each species. One-way analysis of variance was carried out on each variable, standardized for snout-vent length, to assess the degree of interspecific variation. No transformations were necessary since all residuals were normally distributed.

Discriminant function analysis (DFA, method: stepwise) was conducted on a correlation matrix of $\log_e$-transformed data. Analysis was carried out to identify the sex of three specimens for which this information had not been recorded and also to describe the main form of sexual dimorphism for all four species. Inter-species variation was also investigated using DFA. PCA and DFA methods followed those outlined in chapter 3.

Principal co-ordination (using the Gower General Similarity Coefficient) was used to assess phenotypic similarity between species based on combined binomial, categorical and continuous data. Each sex was coded differently for each species to determine the significance of sexual dimorphism within the context of interspecific variability.

Statistical analysis was carried out on Minitab v. 10 for Windows ™ (Ryan *et al.*, 1985), SPSS (10) and MVSP (3.1).

RESULTS

All measurements were found to be highly, or very highly repeatable (see Harper, 1994). Some data points were missing for a number of variables, owing to mechanical damage or disarticulation; in the event of remaining data for these characters not varying significantly between species (one-way analysis of variance), these were removed from subsequent analyses. Where data for one character was missing for up to two specimens of each species, specimens were ordered by size and sex and missing values replaced by the mean of the four surrounding values from that group, using a data replacing function in SPSS. Where data for one character was missing for more than two specimens of each species, that variable was analysed for inter-species variability (significant character variation asterisked in Table 4-4) and removed from subsequent analysis.

Sexual dimorphism

The sex of three *X. vestitus* specimens was not recorded. DFA was conducted on the entire *X. vestitus* sample in order to assess the degree of sexual dimorphism for specimens of known sex, and to attempt to classify unknown cases accordingly. DFA was carried out on all but the first principal component factor to compare shape variation between sexes, PCA1 accounting for size variation within the sample. Results showed that sexual dimorphism in *X. vestitus* was highly significant, and the analysis was able to classify 100% of cases (original and cross-validated) correctly, and also to determine the sex of each of the three additional specimens with a high degree of confidence (Table 4-2).

Inter-specific variation in snout-vent length was highly significant ($F=11.62$, d.f.=3,64, $p=0.000$), with *X. fraseri aff.* measuring significantly less, and *X. l. victorianus* measuring significantly more than the three remaining species. *Xenopus fraseri* was the smallest species, followed by *X. wittei*, *X. vestitus* and *X. l. victorianus* respectively. Having determined the sex of unknown cases, it was also possible to compare snout-vent length between sexes (Table 4-1). The level of significance and direction of variation in snout-vent length between species was the same for separate-sex as for pooled-sex groups, with F values of 20.26 for males, and 9.54 for females. Males are, on average, 20% smaller than females, though this varies inter-specifically.

	X. fraseri aff. n=8m, 9f	*X. wittei* n=8m, 9f	*X. vestitus* n=14f, 5m	*X. l. victorianus* n=7f, 8m
Mean (mm)±s.d. (m&f)	37 ±5	43±4	44±0	49±7
Mean (mm)±s.d. males	34±1	40±2	33±3	44±4
Mean (mm)±s.d. females	42±4	45±4	46±4	53±7
% Difference in mean size between sexes	30%	15%	12.5%	22.5%

Table 4-1

Mean snout-vent length (+/- s.d.) for each taxon, sexes pooled and separate.

Size variation is clearly a significant component of sexual dimorphism. Excluding size from subsequent analysis of sexual dimorphism in order to focus exclusively on shape variation was therefore not justified. Results from size-in DFA analyses are presented (Table 4-2), with F values providing an indication of the relative degree of sexual dimorphism observed for each of the four species, and p values indicating the level of significance of this dimorphism.

	% correct classification with cross validation	Wilk's λ F statistic	d.f.	**p≤0.01 ***p≤0.001
X. fraseri	100	43.78	1,13	***
X. wittei	100	76.2	1,15	***
X. vestitus	94.7	12.77	1,15	**
X. l. victorianus	100	20.1	1,13	***

Table 4-2

Discriminant function analysis- sexual dimorphism. Asterisks refer to the significance level of variation, F values refer to the relative degree of this variation.

DFA listed characters in order of their degree of sexual dimorphism; those accounting for most of the observed variation are listed for each species (Table 4-3). Means and standard deviations of character values (standardized against snout-vent length for all linear measurements) are included to describe the form taken by this variation. Results indicated that the form of sexual dimorphism is inter-specifically variable, a different range of characters accounting for most of the sex-related variation in each species.

Inter-specific variation

One-way analysis of variance was used to assess inter-specific variation for each character, standardized against snout-vent length (Table 4-4). Although sexes were pooled for this analysis, results were cross-validated with those from a subsequent analysis assessing the degree of intra-specific sexual-dimorphism (see below).

A multivariate analysis of shape variation between species was carried out by excluding PCA factor 1 from DFA on remaining factors, PCA 2-8. PCA factor 1 accounted for 52.7% of the total variation, and 50±4% of variation between any one species and any or all of the remaining three. Although character scores were variable in magnitude, all except for two (inter-rtbs dist. and facial flange width between maxillary and pre-maxillary.) were positive. Cross-reference between results from DFA and original PCA character scores gave information about the most discriminatory characters for each species when compared with the remaining three combined (pooled-sex, female and males compared separately) (Table 4-5), and for each species when compared with each of the remaining three species individually (pooled sex only, owing to constraints imposed by sample size) (Table 4-6).

Results from pooled and separate-sex analyses were highly concordant for all species except for *X. l. victorianus.* This high variation in character combinations between individual sex and pooled sex analyses, despite a low F value representing overall sexual dimorphism in the species (Table 4-2), implies that a small number of characters in *X. l. victorianus* are highly sexually dimorphic.

A number of additional osteological characters demonstrated qualitative inter-specific variation, and were scored accordingly. These data were incorporated into individual species osteological accounts.

PCO (Principal Coordinate analysis) was carried out on DFA1-3 (PCA1-5) to give a scatter graph (Figure 4-3) that represents overall phenetic similarity between groups. The differential explanatory power of each of the first three axes, and their corresponding eigenvalues are presented in Table 4-7. Results show *X. wittei* and *X. vestitus* to cluster equidistant from each tetraploid taxon. Some *X. wittei* specimens are indicated as being phenotypically intermediate between tetraploids, whilst the distance separating *X. vestitus* from each tetraploid taxon is approximately equal to the distance separating *X. fraseri* from *X. l. victorianus*. Furthermore, the scatter graph shows overlap between only two of the four taxa, those two being octoploids, *X. vestitus* and *X. wittei.* Sexes do not cluster together within any of these four taxa, indicating that sexual dimorphism is not significant within the context of overall inter-taxon osteological variation.

X. fraseri			*X. wittei*			*X. vestitus*			*X. l. victorianus*		
Char. Number	mean±s.d males	mean±s.d females	Char. Number	mean±s.d males	mean±s.d. females	Char. Number	mean±s.d. males	mean±s.d. females	Char. Number	mean±s.d males	mean±s.d. females
Svl	33.8±1.26	41.73±4.3	89	3.85±.29	4.09±0.21	51	1.09±0.03	1.09±0.03	43	0.4±0.07	0.46±0.03
91	1.64±0.11	1.56±0.09	20	1.19±0.05	1.25±0.08	2	2.24±0.08	2.25±0.16	2	2.67±0.14	2.47±0.21
75	2.5±0.16	2.4±0.2	78	2.79±0.37	3.02±0.25	17b	1.96±0.07	1.94±0.0	76	2.51±0.16	2.61±0.19
92	0.98±0.07	0.88±0.07	73	6.3±0.42	6.67±0.15	52	0.7±0.04	0.7±0.04	75	2.56±0.14	2.54±0.26
76	2.3±0.15	2.3±0.18	82	3.36±0.22	3.32±0.29	svl	33.3±3.0	45.5±3.67	20	1.42±.088	1.36±0.05
1b	2.17±0.18	1.98±0.13	83	3.6±0.2	3.5±0.29	29	0.58±0.06	0.57±0.05	svl	43.57±4.2	53.37±6.7
52	0.77±0.08	0.94±0.08	22	0.38±0.03	0.48±0.09	4	1.08±0.06	0.1±0.08	31	1.15±0.13	1.08±0.06

Table 4-3

Characters demonstrating the greatest degree of sexual dimorphism for each species (identified by DFA). Mean values corresponding to character length (mm [snout-vent length mm]) given for males and females. Key (see also Appendix 1, chapter 3) svl (snout-vent length), 91 (distance between anterior acetabulum margin, pelvic girdle), 75 (length 2nd transverse process), 92 (distance between posterior acetabulum margin, pectoral girdle), 76 (length 3rd transverse process), 1b (maximum width occipitopetrosal), 52 (length maxillary dental ridge), 89 (length iliac shaft), 21 (length frontoparietal posterior from point of association with occipitopetrosal), 78 (length urostyle), 73 (length vertebral column), 82 (femur length), 83 (length fibio-tibula), 22 (length from tip of frontoparietal to pineal foramen), 51 (depth dental arch), 2 (maximum length skull), 17b (length from tip of frontoparietal to foramen magnum), 29 (width foramen magnum), 4 (maximum width nasal), 43 (length squamosal arm), 31 (distance separating ventral occipitopetrosal knobbles).

Character number	Character	F	*p=0.05, **p=0.01, ***p=0.001	Tukey	Mean (cm), standard deviation			
					1 *X. fraseri*	2 *X. wittei*	3 X. *vestitus*	4 X. *l. victorianus*
1b Maximum width occipitopetrosal		6.46	**	3(1,2,4)	2.1±0.18	2.1±0.23	1.9±0.08	2±0.2
2 Maximum length skull		5.28	**	3(1,2,4)	2.5±0.4	2.5±0.3	2.25±0.13	2.6±0.2
3 Width nasal at point of association with frontoparietal		18.9	***	3(1,2,4)	0.9±0.1	0.85±0.07	0.72±0.05	0.87±0.08
4 Maximum width nasal		6.12	**	1(3,4)	1.25±0.18	1.18±0.09	1.09±0.07	1.12±0.1
17b Frontoparietal length		3.36	*	1(3)	2.1±0.16	2.07±0.13	1.95±0.09	2.06±0.19
19 Width frontoparietal at level of occipitopetrosal		16.74	***	3(1,2,4), 2(3)	1.01±0.12	0.89±0.08	0.79±0.05	0.87±0.1
20 Length frontoparietal anterior from level of association with occipitopetrosal		11.37	***	1(2,3), 4(2,3)	1.42±0.2	1.22±0.08	1.2±0.15	1.39±0.07
21 Length frontoparietal posterior from level of association with occipitopetrosal		3	*	2(3)	0.7±0.14	0.79±0.15	0.68±0.06	0.69±0.15
22 Length frontoparietal anterior from pineal foramen		10.55	***	1(2,3), 2(4)	0.63±0.15	0.43±0.09	0.49±0.08	0.58±0.11
23 Length frontoparietal from anterolateral alae to pineal foramen		7.59	***	1(2)	0.31±0.097	0.195±0.08	0.24±0.08	0.32±0.1
24 Length frontoparietal posterior from pineal foramen		4.1	*		1.52±0.16	1.63±0.18	1.45±0.11	1.59±0.2
29 Width foramen magnum		4.94	**	1(3), 2(3)	0.63±0.06	0.6±0.07	0.57±0.05	0.58±0.06
30 Depth foramen magnum		8.59	***	1,4(2,3)	0.29±0.08	0.39±0.07	0.37±0.07	0.29±0.06
31 Distance between ventral occipitopetrosal nobbles		11.81	***	3(1,2,4)	0.22±0.2	1.15±0.09	0.98±0.08	1.11±0.102
34 Width vomer		9.45	***	1(3,4), 2(4)	0.55±0.09	0.51±0.05	0.56±0.05	0.44±0.07
36 Anterior width sphenethmoid		35.75	***	3(1,2,4)	0.74±0.08	0.79±0.09	0.58±0.04	0.79±0.07
40 Inter-rtbs distance		48.48	***	1,2(3,4)	0.27±0.05	0.31±0.09	0.13±0.04	0.11±0.04
41 Rostral width columella		15.57	***	1(2,3), 2(3,4)	0.098±0.02	0.13±0.04	0.07±0.01	0.079±0.02
42 Length columella		5.96	**	3(1,2,4)	0.67±0.12	0.65±0.09	0.57±0.05	0.69±0.09
43 Length squamosal arm		12.29	***	3(1,2,4)	0.44±0.11	0.44±0.11	0.6±0.08	0.43±0.06
44 Length tympanic annulus		8.42	***	1(3), 2(3)	0.66±0.14	0.77±0.22	0.53±0.06	0.64±0.1

47 Length anterior ramus tympanic annulus	10.64	***	1(2,3), 3(4)	0.17±0.04	0.23±0.08	0.27±0.06	0.2±0.04
50 Length premaxillary	12.29	***	1(3), 2(3,4)	0.37±0.04	0.39±0.04	0.32±0.03	0.35±0.04
51 Length maxillary	8.95	***	1(3,4), 2(3,4)	1.19±0.14	1.22±0.09	1.09±0.09	1.07±0.1
52 Length maxillary dental row	11.18	***	1(4), 2(3), 3(4)	0.76±0.08	0.83±0.12	0.7±0.04	0.85±0.08
53a Depth dental arch	7.87	***	1(3,4), 2(4)	1.11±0.17	1.1±0.09	1±0.06	0.94±0.07
53b Depth maxillary arch	4.38	**	2(4)	1.19±0.37	1.31±0.11	1.19±0.06	1±0.11
54 Number of premaxillary teeth	6.33	**	1(4), 3(4)	6.5±0.8	6.8±1.3	6.3±0.8	7.63±0.79
55 Number maxillary teeth	15.05	***	4(1,2,3), 1(3), 2(3)	13.5±1.9	16±3.2	13.9±1.6	18.3±2
56 Width facial flange at maxillary/premaxillary junction	10.99	***	4(1,2,3)	0.1±0.03	0.13±0.04	0.1±0.03	0.06±0.03
62 Inter-otic gap	8.94	***	1(3,4)	0.7±0.18	0.6±0.09	0.51±0.07	0.51±0.01
63 Inter-pterygoid base distance	46.58	***	3(1,2,4)	1.4±0.14	1.3±0.12	0.98±0.08	1.3±0.13
67 Length anterior pterygoid ramus	12.37	***	3(1,2,4)	0.55±0.09	0.55±0.06	0.45±0.07	0.59±0.05
73 Length vertebral column	16.41	***	3(1,2,4)	6.23±0.5	6.5±0.3	5.7±0.3	6.5±0.2
74 Width transverse process I	15.09	***	1(2,3,4), 2(3)	1.85±0.14	1.7±0.1	1.58±0.1	1.65±0.16
75 Width transverse process II	25.28	***	3(1,2,4)	2.4±0.19	2.34±0.14	2.07±0.15	2.55±0.18
76 Width transverse process III	39.35	***	3(1,2,4), 4(2,3)	2.29±0.16	2.2±0.2	1.94±0.13	2.56±0.18
77a Length sacral diapophysis	11.34	***	1(2,4), 2(3), 4(3)	1.88±0.26	2.14±0.19	1.8±0.15	2.12±0.23
77b Width sacral diapophyses	8.71	***	1(2,3), 3(4)	1.82±0.16	1.69±0.12	1.62±0.13	1.8±0.11
78 Length urostyle	8.83	***	3(1,2,4)	3±0.4	2.9±0.3	2.58±0.25	3.09±0.23
82 Femur length	8.02	***	1(2,3)	3.74±0.33	3.34±0.2	3.34±0.2	3.52±0.3
83 Fibiale-tibula length	18.92	***	1(2,3,4)	4.17±0.34	3.56±0.25	3.56±0.25	3.75±0.34
84 Fibiae-tibulare length	7	***	1(2,3)	2.3±0.3	2.04±0.14	2.06±0.16	2.13±0.21
85 Length tarsus II	19.7	***	1(2,3,4)	3.7±0.38	4.1±0.34	3.4±0.14	4.1±0.3
86 Length humerus	11.08	***	3(1,2,4)	2±0.2	1.94±0.17	1.66±0.11	1.94±0.25
87 Length radio-ulnare	10.03	***	3(1,2,4)	1.6±0.2	1.6±0.17	1.32±0.12	1.63±0.28
88 Length carpus II	54.1	***	3(1,2,4)	1.78±0.16	1.69±0.1	1.28±0.1	1.8±0.2
89 Length iliac shaft	3.02	*	2(3)	3.9±0.3	3.9±0.3	3.7±0.2	3.88±0.5
91 Distance between anterior acteabular margins	5.17	**	1(3)	1.59±0.1	1.48±0.11	1.42±0.13	1.5±0.2
94 Medial width clavicle	37.45	***	1(2), 2(3), 4(1,2,3)	0.39±0.07	0.52±0.09	0.42±0.08	0.73±0.14
96 Scapula length	8.07	***	2(1,3,4)	0.48±0.06	0.55±0.06	0.47±0.05	0.48±0.05

100 Length distal prehallux	35.09	***	1(2,3,4)	0.4±0.04	0.3±0.05	0.27±0.04	0.27±0.04
102 Length ultimate phalanx tarsus II	3.67	*	1(3)	0.34±0.04	0.34±0.03	0.29±0.004	0.32±0.05
103 Length prepollex	10.63	***	1(4), 2(3), 3(4)	0.11±0.02	0.12±0.02	0.09±0.02	0.15±0.03

Table 4-4

Characters and their corresponding numbers (listed in Appendix 1, chapter 3) identified by one-way analysis of variance as showing significant inter-taxon osteological variation (Degrees of freedom, 3, 64). Asterisks refer to significance levels. Numbers 1-4 apply to each of taxa *X. fraseri, X. wittei, X. vestitus* and *X. l. victorianus* respectively. The character in taxa (taxon number) preceding brackets differs significantly in length (number, in the case of teeth) from those enclosed by brackets. Mean values correspond to linear measures and s.d. for each character. Abbreviations: rtbs: retractor bulbi muscle scars

Species	*X. fraseri*			*X. wittei*			*X. vestitus*			*X. l. victorianus*		
Sex	M&F	F	M	M&F	F	M	M&F	F	M	M&F	F	M
F	87.92	63.3	8.8	66	36.3	167	42.3	20.1	22.1	33.8	6.66	4.6
d.f.	1,66	1,37	1,26	1,66	1,37	1,26	1,66	1,37	1,26	1,66	1,37	1,26
p	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.04
%	96%	95%	89%	94%	88%	100%	97%	92%	100%	85%	74%	86%
1	47	47	47	40	40	41	47	47	47	40	101	41
2	30	30	30	44	41	40	30	30	30	56	99	40
3	100	100	43	43	23	44	100	100	12	39	96	44
4	43	22	54	22	90	22	43	22	54	34	89	22
5	22	43	55	23	22	23	22	43	55	100	103	23

Table 4-5

Inter-specific variation in osteology. Each taxon was compared with remaining three taxa (combined) using DFA. Sexes were compared together (M&F) and separately (M, F). Results from one-way analysis of variance (F, d.f., p), comparing DFA1 values (M&F, M, F) between taxa, are given. Correct classification rates (%) are also listed. The five most discriminatory characters for each taxon (M&F, M, F) are listed in order of importance, by column, in the lower half of the table.
Key (see also Appendix 1, chapter 3): 47 (length anterior squamosal ramus), 30 (depth foramen magnum), 100 (length distal prehallux), 43 (length squamosal arm), 22 (length from tip frontoparietal to pineal foramen), 54 (number premaxillary teeth), 55 (number maxillary teeth), 40 (inter-rtbs gap), 44 (length tympanic annulus), 23 (distance from anterolateral frontoparietal alae to pineal foramen), 41 (anterior width columella), 90 (length ischium), 12 (distance between anterior septomaxillary and nasal), 56 (width facial flange at anterior maxillary), 39 (diameter optic foramen), 34 (maximum width vomer), 101 (length penultimate phalanx, tarsal II), 99 (length proximal prehallux), 96 (length scapula), 89 (length iliac shaft), 103 (length prepollex).

	X. wittei (F, d.f., p, %)	*X. vestitus* (F, d.f., p, %)	*X. l. victorianus* (F, d.f., p, %)
X. fraseri aff.	34; 1,32; 0.000; 85%	176.8; 1,34; 0.000; 92%	11; 1,30; 0.002; 69%
Character no	22,41,100,44,23,83,56	40,100,47,30,43,19,41	100,40,44,39,43,34,103
X. wittei		40.59; 1,34; 0.000; 83%	19; 1,30; 0.000; 91%
Character no		41,43,40,22,44,23,83	40,51,36,56,34,96,103
X. vestitus			10.67; 1,31; 0.003; 67%
Character no			43,47,56,30,34,39,51

Table 4-6

Inter-specific variation in osteology. Each taxon was compared with remaining three taxa (combined) using DFA - Pooled-sex only. Results from one-way analysis of variance (F, d.f., p) comparing DFA1 values between taxa are given. Correct classification rates (%) are also listed. The seven most discriminatory characters for each taxon are listed in order of importance. Key (see also Appendix 1, chapter 3): 83 (length fibio-tibula), 19 (width frontoparietal at point of association with occipitopetrosal), 51 (length dental ridge), 36 (anterior width sphenethmoid). All other characters are listed below Table 4-5.

	Axis 1	Axis 2
Percentage	50.4	30.8
Cumulative Percentage	50.4	80.2

Table 4-7

Principal coordinate analysis- percentage (and cumulative percentage) variations explained by axes 1-3.

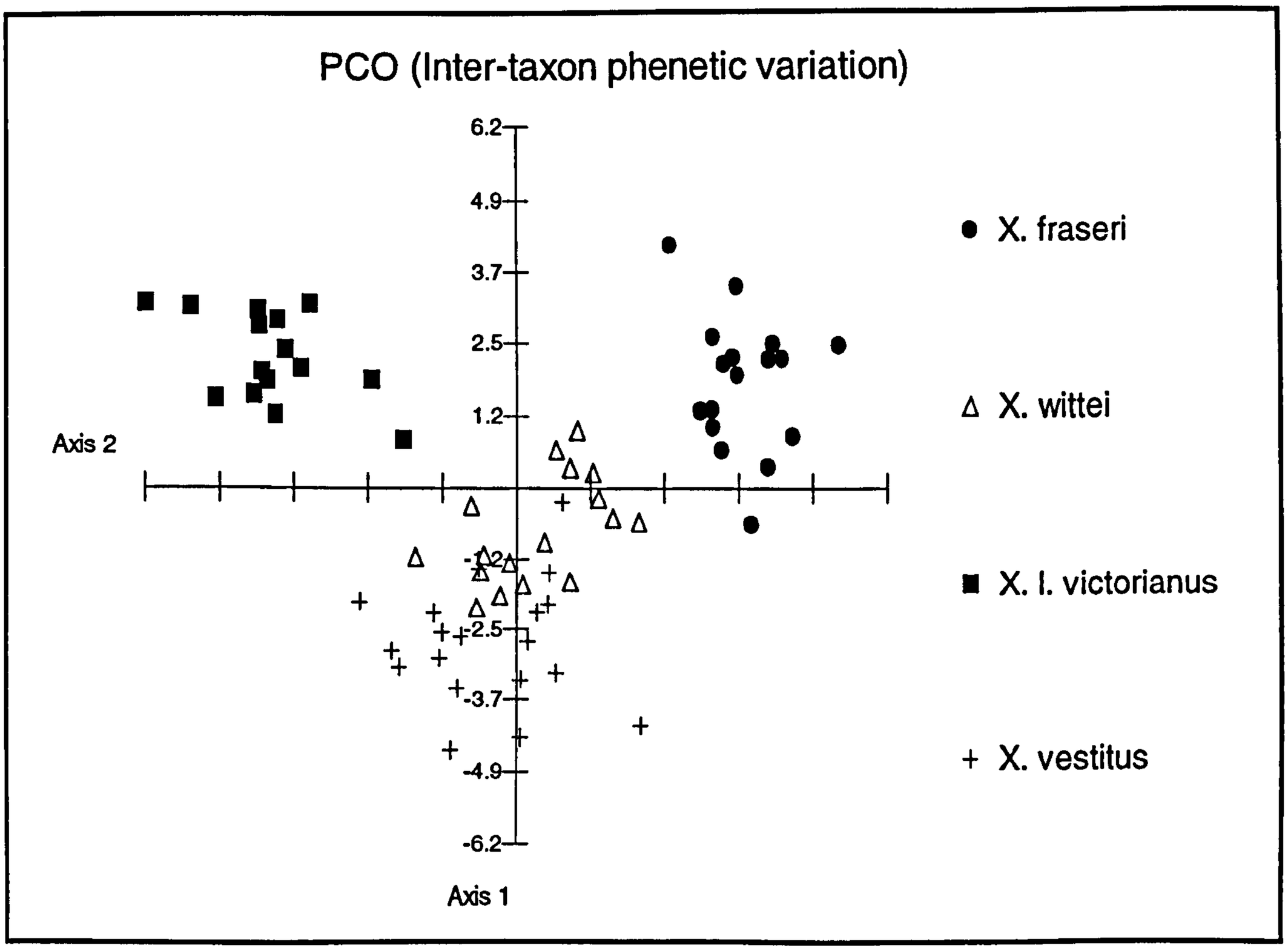


Figure 4-3

Scatter plot based on similarity coefficients (Euclidean measure of distance): PCO on DFA 1-3 (of PCA 1-5). Sexes pooled per taxon. The *X. wittei* cluster is approximately intermediate between tetraploids *X. fraseri* and *X. l. victorianus*. The *X. vestitus* cluster is equidistant from each tetraploid cluster, but is separated by a greater phenotypic distance from each than is *X. wittei*. Octoploid clusters are shown to partially overlap along both axes, but are separated along a third, not shown here.

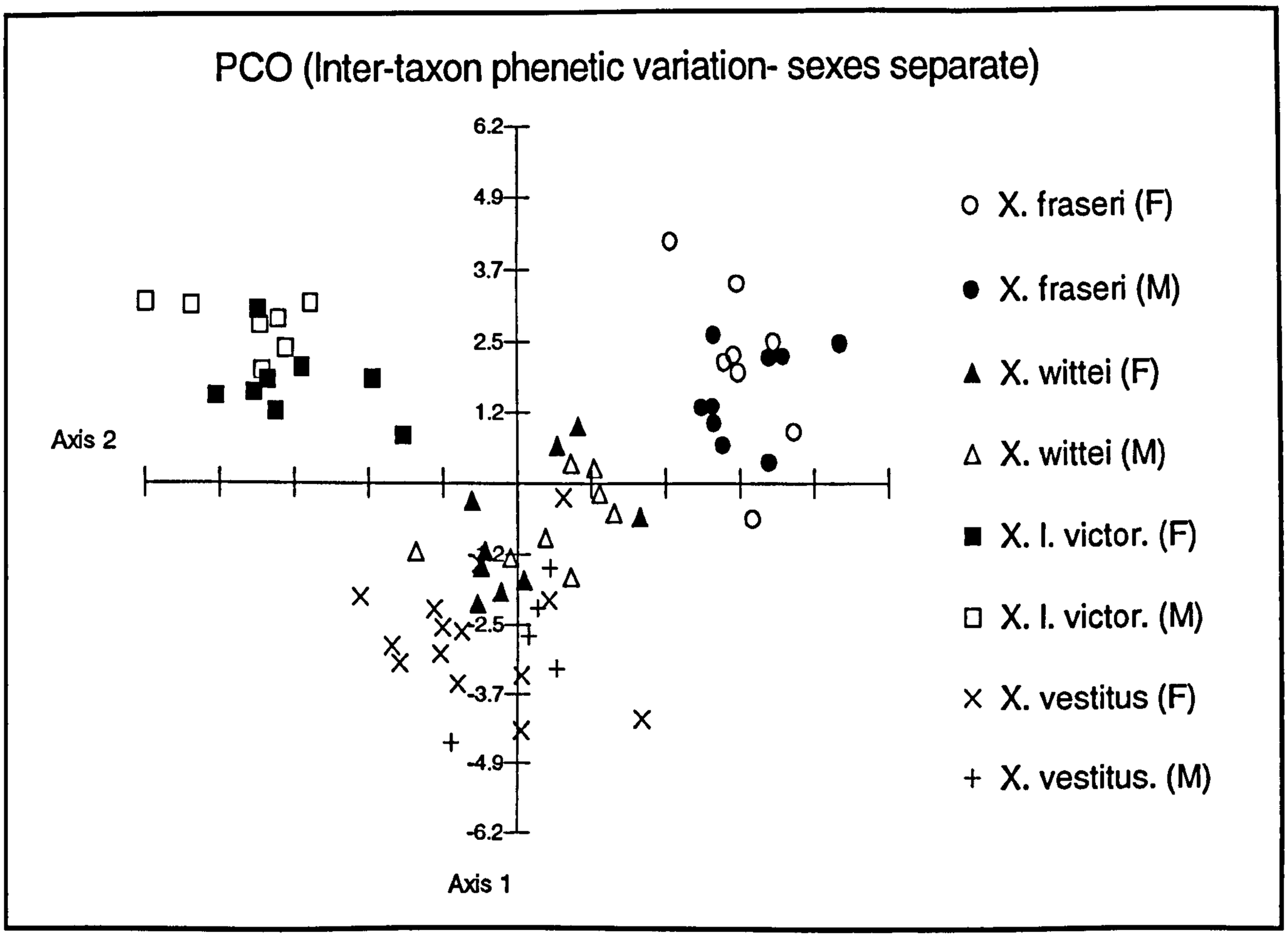


Figure 4-4

Scatter plot based on similarity coefficients (Euclidean measure of distance): PCO on DFA 1-3 (of PCA 1-5). Sexes plotted separately for each taxon.

DESCRIPTIONS OF THE OSTEOLOGY OF *X. FRASERI, X. L. VICTORIANUS, X. WITTEI AND X. VESTITUS*.

Xenopus fraseri- Osteological description

Cranium

Frontoparietal

This short, broad element is parallel-sided, widening abruptly at its lateral point of association with occipitopetrosals (Illustration 4-1). Caudally, the element is rounded and lacks posterolateral alae. Anterior margins are straight, form an obtusely-angled apex and meet lateral margins to form distinct, but unmodified anterolateral corners. The skull table is flat, wide, and bordered either by sigmoid or straight parasagittal crests that diverge anteriorly and converge on a narrow U-shape posterior margin, just short of the foramen magnum. In the case of sigmoid crests, the skull table widens from its posterior margin to the level of cerebral hemispheres, and diverges once again towards anterolateral corners. The pineal foramen is positioned approximately one third of the way along the element.

Sphenethmoid

The main body of the element is usually parallel-sided, but in some specimens, may widen slightly towards the anterior margin. In the majority of specimens, orbitonasal foramina are represented by laterally displaced notches that occur along the anterior margin of the element (Illustration 4-2). In some however, these foramina are completely enclosed in bone.

Retractor bulbi muscle scars (rtbs) are widely separated and enclose small, laterally positioned optic foramina. Scars extend towards posterolateral alae, which are no wider than the main sphenethmoid body. Prootics are partially bordered by these alae and are particularly small. The caudal sphenethmoid process is rhomboid-shaped, extending almost as far as the foramen magnum margin. The vomer is edentous, usually large and azygous, but may occasionally be paired or fractured.

Occipitopetrosal

Anterior margins are perpendicular and posterior margins oblique, with respect to the main body axis (Illustration 4-1, Illustration 4-2). They converge on narrow crista parotica, which though somewhat variable, are generally short. Occipitopetrosals are deep relative to their width, and their posterior faces are convex. Medially, they are fused around the wide foramen magnum. Dorsal crests are well-developed and occasionally bear a single, short, posteromedially-directed branch. Eustachian troughs are broad and bordered posteriorly by a bony ridge that extends posteromedially towards the

widest point of the caudal sphenethmoid process. Prominences along this ridge vary extensively in both size and shape.

Pterygoid

The proximal flange of the anterior ramus is uncleft ventrally, and distal flange only moderately curved (Illustration 4-2). Lateral rami lack any distinguishing features. Medial rami, or otic plates are bifid, the smaller, anteromedially-directed flange is tapered whilst the larger, medially-directed ala is irregularly tapered and the orientation of its posterior margin is perpendicular to sub-perpendicular with respect to the zonal axis. The inter-otic gap is wide, measuring approximately the same as the main sphenethmoid body.

Tympanosquamosal

The annulus lacks any form of otic process to support the columella at its pivot point (Illustration 4-1). The squamosal is long and moderately robust, extending to articulate with the dorsal face of the anterior-most pterygoid flange by means of a well-defined zygomatic ramus. The junction between squamosal and annulus varies between being deeply V-shaped to broadly U-shaped. At its base, the tympanosquamosal forms a short, broad stem that is separated from the narrow cristae parotica by a cartilaginous bridge. The columella is considerably longer anterior than posterior to its pivot point with the tympanosquamosal, a reflection on the shortness of both fenestra ovalis and crista parotica. The shaft is moderately wide.

Maxillary Arch

The distally tapered maxillary arch is moderately long, extending approximately half way across the orbit, but not beyond the distal flange of the anterior pterygoid ramus (Illustration 4-2). The dental row extends along the entire length of pre-maxillaries and along between one half and two thirds the length of maxillaries. Unusually, there is relatively little overlap between partes palatina of these paired elements. Partes palatina and facialis are relatively broad, giving the element a robust appearance.

Nasal and Septomaxillary

The nasal is short and wide (Illustration 4-1). The rostral extension is occasionally slightly bulbous, unscarred, uncleft and reaches the anterior margin of the skull. Anterolateral margins of the nasal are usually oriented at an angle perpendicular to the main body axis, and lacrimonasal foramina are absent from lateral extensions. In some specimens, anterolateral margins are oriented at a slightly acute angle with respect to the main nasal axis. Septomaxillaries are long and lack a lacrimonasal foramen. They are more widely positioned than in other taxa.

Mandible

As for chapter 2.

Calcification

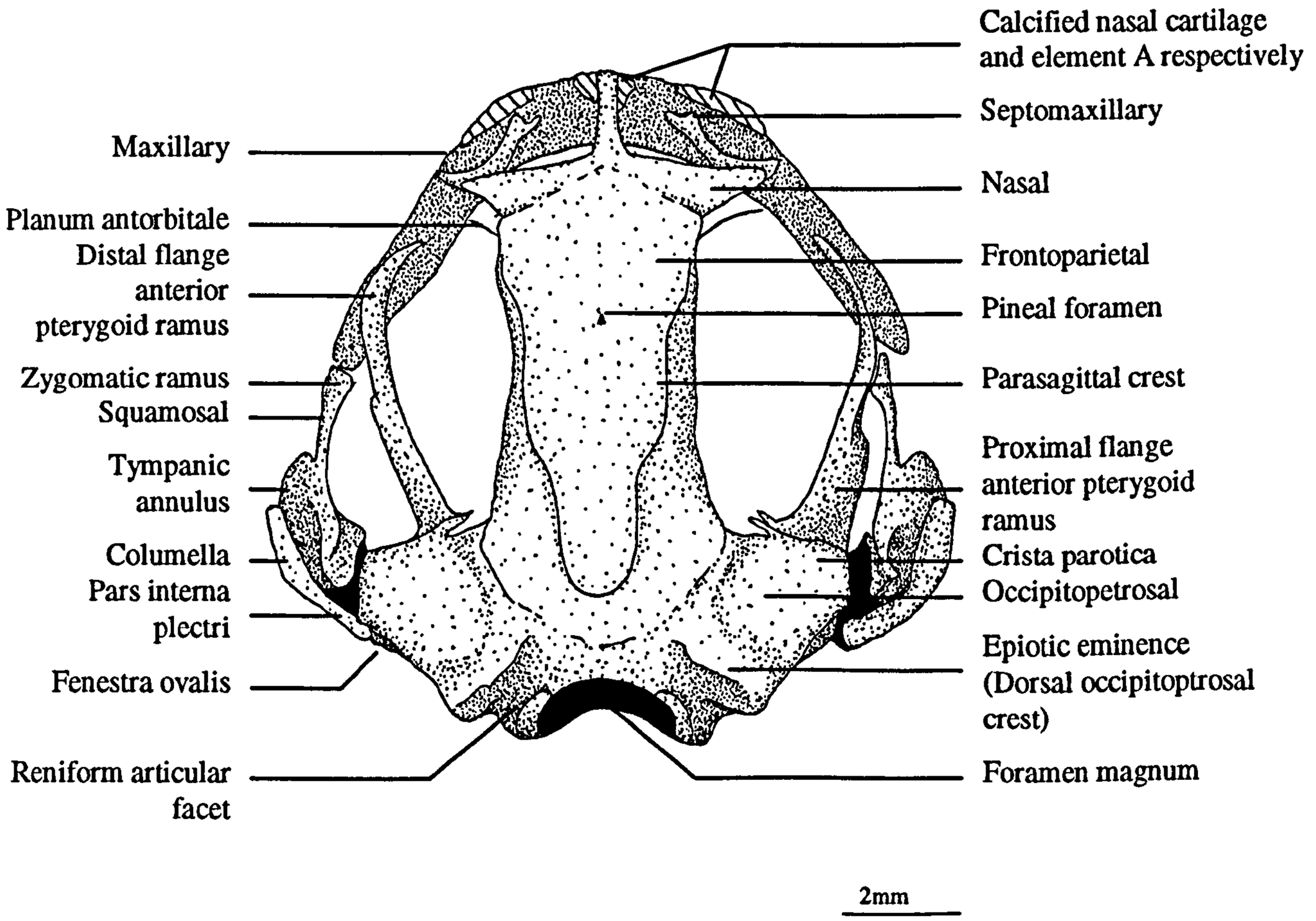
All rostral cranial elements demonstrate an extreme degree of calcification. Prevalence of calcification of the planum antorbitale is low however, compared with other cranial elements.

Fusion

Fusion between adjacent elements is extensive, occurring between occiptopetrosals medially, frontoparietal and occipitopetrosal, frontoparietal and nasal, frontoparietal and sphenethmoid, laterally, sphenethmoid and occipitopetrosal, and between vomer and sphenethmoid.

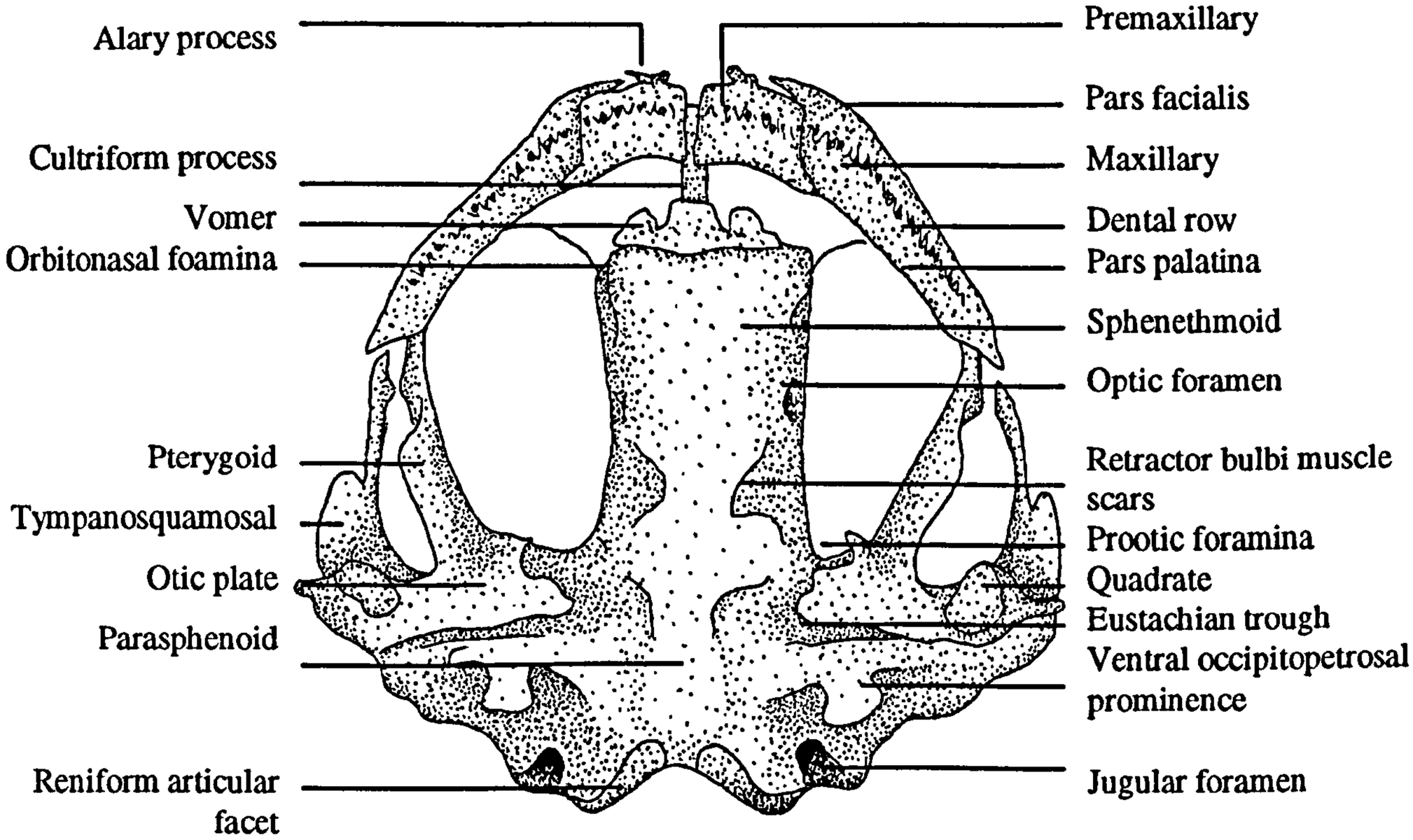
Hyolaryngeal apparatus

As for chapter 2.



Dorsal view *X. fraseri* skull

Illustration 4-1



Ventral view *X. fraseri* skull

Illustration 4-2

Axial skeleton

The vertebral column is long and narrow, as determined by widths of vertebrae, transverse processes and sacral diapophyses relative to column length (Illustration 4-3, Illustration 4-4). The pre-sacral portion far exceeds the post-sacral portion in length. The atlas bears a straight anterodorsal margin, which, in most specimens, narrows towards a posterior margin that bears widely separated, paired, dorsal spines. All subsequent vertebrae range from being un-ornamented with slightly convex posterior margins to moderately ornamented with single dorsal spines. Zygapophyses are generally positioned within the lateral margin of the column, but sometimes occur on the outside margin. The first pair of transverse processes is oriented perpendicularly or anterolaterally with respect to the main body axis, is widest midway along its length, or may occasionally be parallel-sided, tapering distally. They are approximately the same in width as the atlas. The second pair measures approximately 140% the length of the first, is anterolaterally-oriented on leaving the column, curving posterolaterally; curvature is mainly proximal. The third pair of transverse processes measures approximately 130% the first and leaves the vertebral column at right angles. Curvature is slightly greater than that of the second pair and is also mainly proximal. Remaining transverse processes conform to the description given in chapter 2 but are particularly short. Ventrally, the atlas bears a convex anterior margin that is medially notched in a high proportion of specimens. Articular faces between centra are moderately narrow with correspondingly large spinal foramina separating pedicels. In most specimens, sacral diapophyses are parallel-sided and asymmetrical around the transverse plane, being longer caudally. Vestigial processes are absent from the urostyles of most specimens.

Variation, oddities and deformities

One specimen bears bilateral processes along the anterior margin of sacral diapophyses. Atlas and pre-sacral II are fused in two specimens, adjacent transverse processes incorporated into this synostosis in the former.

Anterior Appendicular Skeleton

Pectoral girdle

Suprascapular and cleithrum are small, and do not overlap mid-dorsally (Illustration 4-5). Paired alae of the cleithrum are relatively robust, the cleft separating them not as deep as in most other taxa (*X. muelleri* and *X. laevis*), and the anteriormost flange, despite being the smaller of the two, is relatively broad compared with those of other taxa. Partes acromialis and glenoidialis are usually completely fused, however in some specimens, distal margins fuse creating a proximal foramen between partes. Clavicles arch anteromedially, their medial margins, expanded to varying

degrees, measuring between one and three times the proximal shaft width. Lateral and medial margins of coracoids are approximately equal in width; the gap that separates them medially measures approximately twice the medial width of the element. Prevalence of calcification of epicoracoid, suprascapular and sternal cartilage though variable, is generally high.

Forelimb

As for account in chapter 2. Phalanges are short.

Posterior Appendicular Skeleton

Pelvic girdle

Iliac crests extend anteriorly from their broad, U-shaped inter-iliac symphysis, and whilst they are parallel in most specimens, in others ilia diverge slightly. Elements are synostotically fused with no trace of scars.

Hindlimb

All elements conform to the description in chapter 2. The distal prehallical element measures approximately three times the length of the proximal element, is generally triangular in shape (flat transverse section), and in some cases bears a small shelf along the inner margin, which serves as a holdfast for the 4th metatarsal claw.

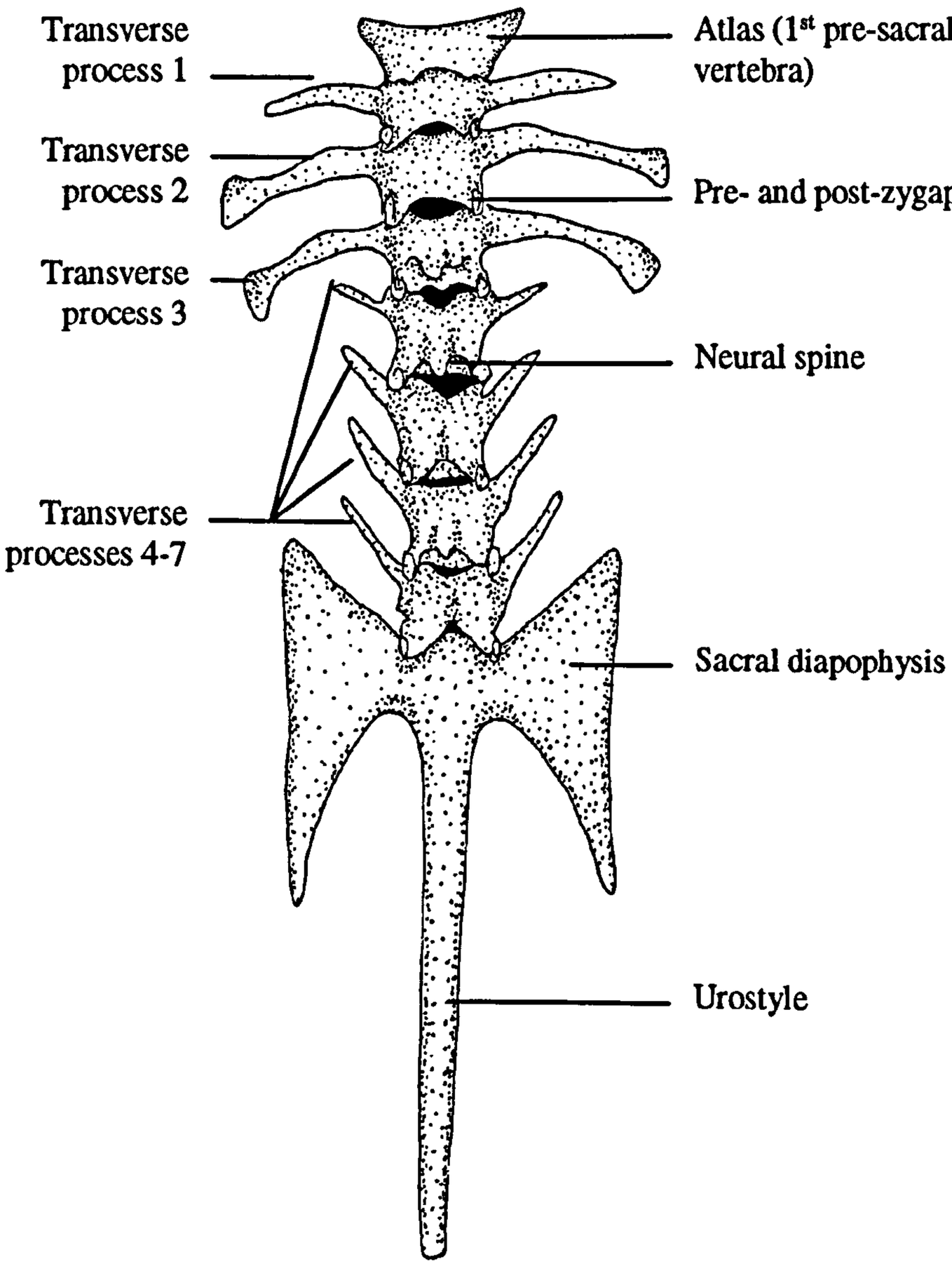
Sexual Dimorphism

The main osteological manifestation of sexual dimorphism is in gross body length (snout-vent length), with males being approximately 30% shorter than females. Males are characterized by having a wider pelvic girdle at the acetabular level, a longer second pair of transverse processes relative to first and third pairs, broader skull at the level of cristae parotica and a tympanic annulus that is more dorsally-oriented than in females. Males also have shorter maxillary tooth rows than females.

Principal features that distinguish X. fraseri *from the remaining three taxa in the analysis*

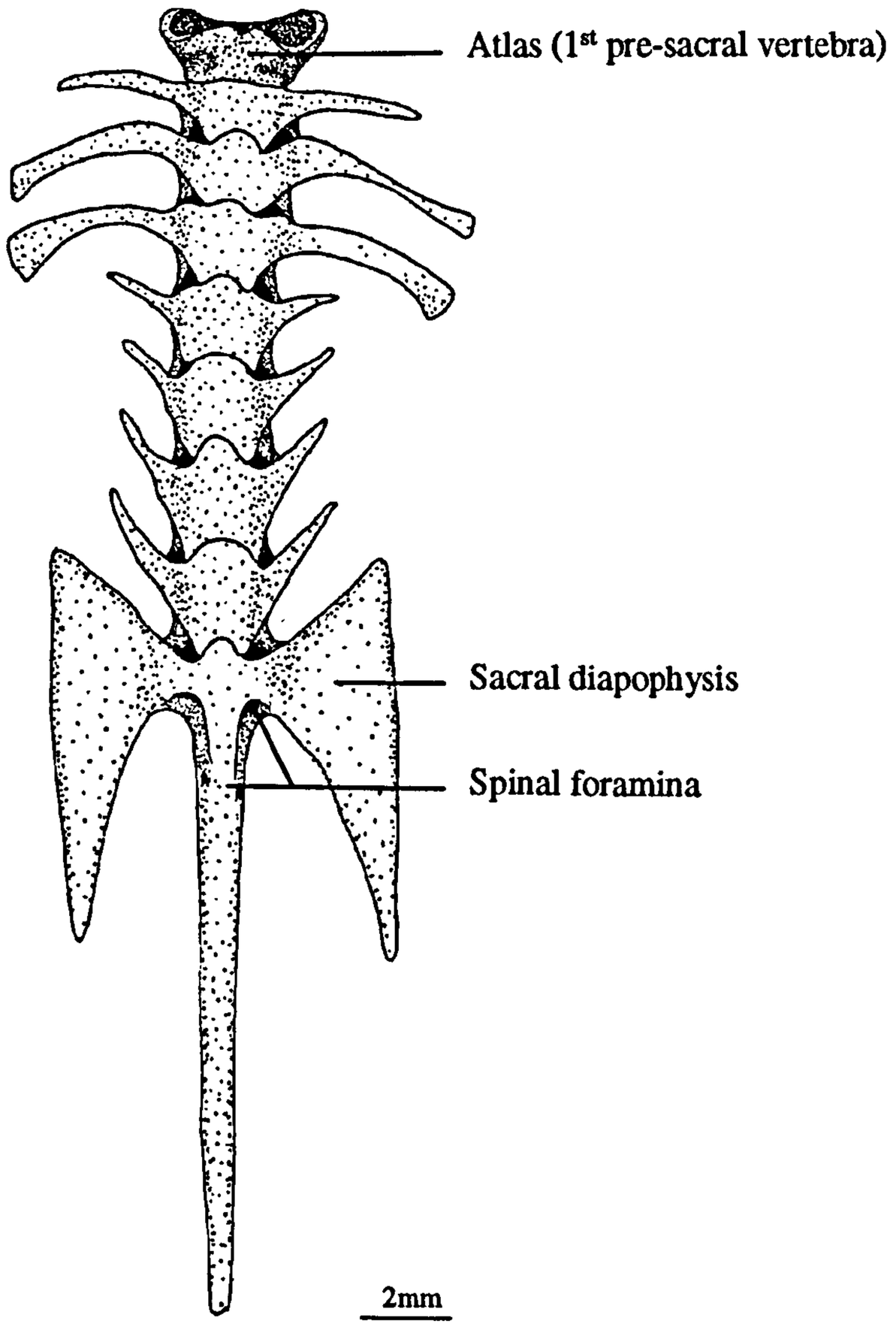
The frontoparietal is parallel-sided, crests diverging, sigmoid-shaped or straight, but not parallel. The pineal foramen is positioned caudally, the vomer large. Rtbs are widely separated, and a high degree of fusion connects adjacent elements. The inter-otic gap is moderately wide and there is no overlap between tympanum base and occipitopetrosals. The columella is longer anterior than posterior to pivot point and has a narrow shaft. The anterior pterygoid ramus is short, and maxillary arch long and tapered, despite having a relatively short maxillary dental row. The nasal is short and wide. A high degree of calcification characterizes anterior cranial elements (except planum antorbitale). The foramen magnum bears a shallow, dorsal intercotylar notch. The vertebral column is

long and narrow, the pre-sacral exceeding post-sacral portion in length. Dorsal spines on atlas are widely separated and paired. Transverse processes are moderately straight, a small distal cartilaginous cap occurring on the third pair. Cleithrum and suprascapula are small and scapula long. A shallow cleft separates partes of cleithrum. A high mean angle formed between zonal plane and coracoid. Finally, the distal-to-proximal prehallux length ratio is relatively high.



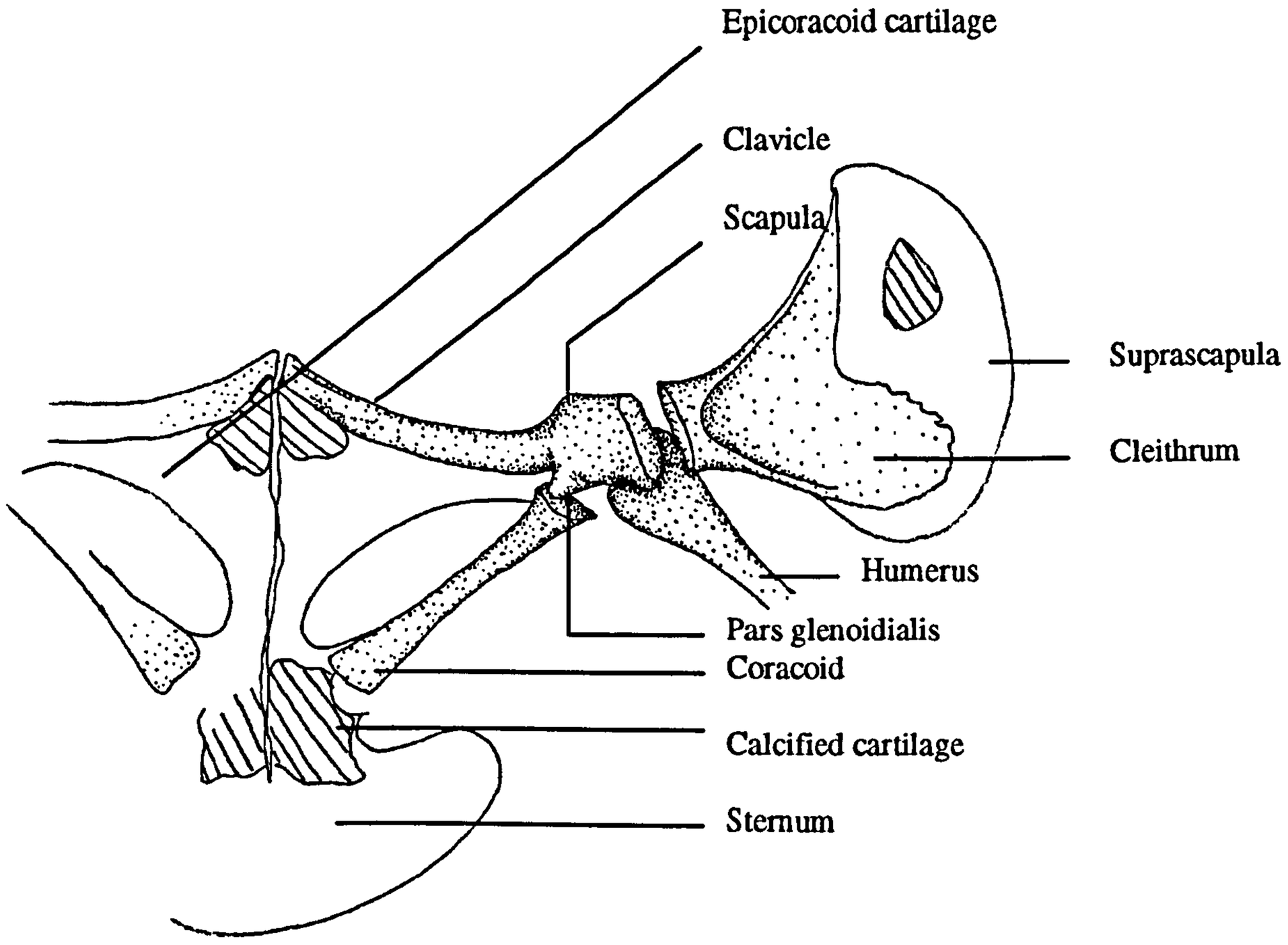
Dorsal view *X. fraseri* vertebral column

Illustration 4-3



Ventral view *X. fraseri* vertebral column

Illustration 4-4



Pectoral girdle *X. fraseri*

Illustration 4-5

Xenopus laevis victorianus- Osteological Description

Cranium

Frontoparietal

This hourglass- or parallel-shaped element is approximately equal in width anterolaterally as at its point of association with occipitopetrosals (Illustration 4-6). It is narrowest just anterior from this site, widening towards anterolateral corners. Caudally the profile is either U-shape or square and lacks distinct posterolateral alae. Anterior margins are straight or concave, forming an obtuse apex. The skull table, as such, is lacking in most specimens, parasagittal crests instead forming a medial ridge that extends from just within the posterior margin, to the level of anterolateral corners where crests diverge slightly. Occasionally however, crests are not fully converged, and a skull table measuring up to one-third the width of the element forms, especially in the case of males. The pineal foramen is positioned approximately a quarter of the way along the element from the apex tip.

Sphenethmoid

The sphenethmoid is parallel-sided. Its anterior margin sometimes bears a pair of laterally displaced notches that represent orbitonasal foramina (Illustration 4-7). Rtbs are separated by a narrow gap and enclose optic foramina, which are small and laterally-displaced. Scars extend toward posterolateral alae, which vary in size but are no more widely separated than the main sphenethmoid body. The crests seen connecting these scars caudally in some other taxa are absent in all but one of these specimens. Beyond these alae, margins converge to form a broad, spatulate process that is either parallel sided or widest at its posterior margin, and extends just short of the foramen magnum. The vomer is moderate in size, edentulous and azygous in the majority of specimens, and is cylindriform or rhomboid in shape.

Occipitopetrosal

Anterior margins are perpendicular and posterior margins oblique with respect to the main body axis, converging laterally on well-developed, long, narrow crista parotica (Illustration 4-6, Illustration 4-7). Medially, elements are partly fused around the narrow foramen magnum. Dorsal crests are well-developed in most specimens, giving the element an appearance of bilateral concavity. Ventrally, broad Eustachian troughs extend posteromedially towards the widest point on the caudal sphenethmoid process.

Pterygoid

The proximal flange of the anterior ramus is long and uncleft, and the distal flange short and moderately-to-well curved (Illustration 4-6, Illustration 4-7). Lateral rami lack any distinguishing

features. Otic plates are asymmetrically bifid. The anterolaterally directed, smaller flange is tapered to a point and extends anteromedially around the pterygoid knob, whilst the medially directed, main process is long, slender and unevenly tapered. A small inter-otic gap separates plates medially and posterior margins are anteromedially oriented, covering approximately half the Eustachian trough.

Tympanosquamosal and columella

The annulus is short and round with a long, moderately slender squamosal that articulates by means of a poorly defined zygomatic ramus with the posterior face of the distal flange of the anterior pterygoid ramus (Illustration 4-6, Illustration 4-7). In most cases, the site of divergence between annulus and squamosal is deeply cleft, though it may also be U-shaped. The tympanum lacks well-developed otic rami to support the columella at its pivot point, and is supported at its base by a well-developed stalk that does not overlap the crista parotica. The columella is approximately equal in length anterior and posterior from its pivot point with occipitopetrosals.

Maxillary arch

Maxillaries are short and semi-tapered, distally (Illustration 4-7). They extend between a quarter and one third of the way across the orbit, and do not extend beyond the posterior margin of the anterior-most pterygoid flange. The dental row extends the length of pre-maxillaries and four-fifths the length of maxillaries. Partes palatina and facialis are slender to moderate and alary processes bifid.

Nasal and Septomaxillary

The rostral nasal extension is parallel-sided, unscarred and uncleft with a moderate degree of ventral curvature (Illustration 4-6). Anterior margins of lateral extensions are either straight or sigmoid-shaped, and are oriented at an angle perpendicular to the main body axis in most specimens, but may occasionally be posterolaterally oriented. Lateral foramina are absent, and in some specimens, bilateral prominences occur in the most concave portion of the nasal. Septomaxillaries are short and oriented at a low angle with respect to the main body axis.

Mandible

As for account in chapter 2.

Calcification

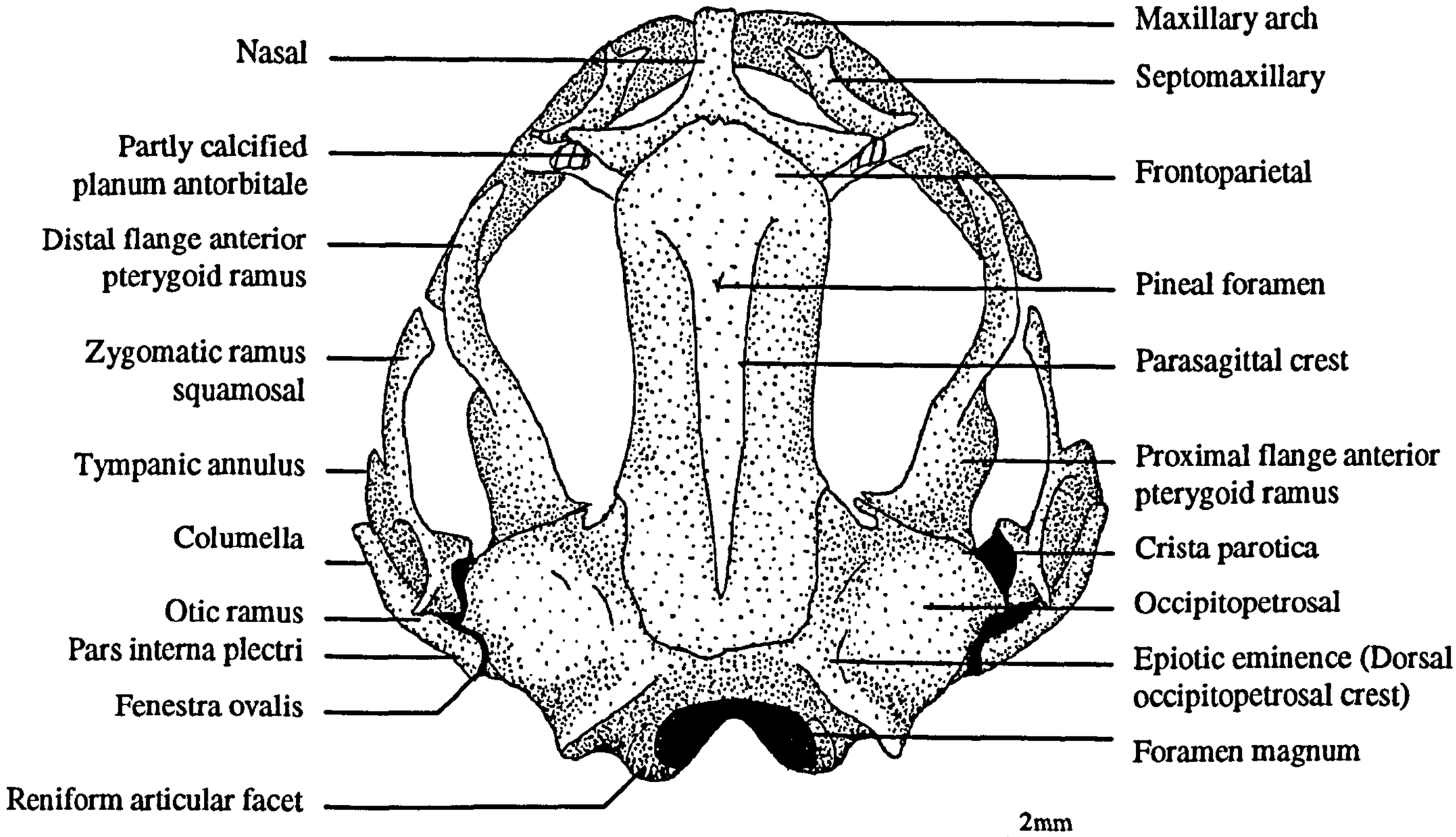
Calcification of all rostral cartilaginous elements is generally very high.

Fusion

The degree of fusion between cranial elements is low, and limited to partial fusion between the occipitopetrosal, frontoparietal and/or sphenethmoid.

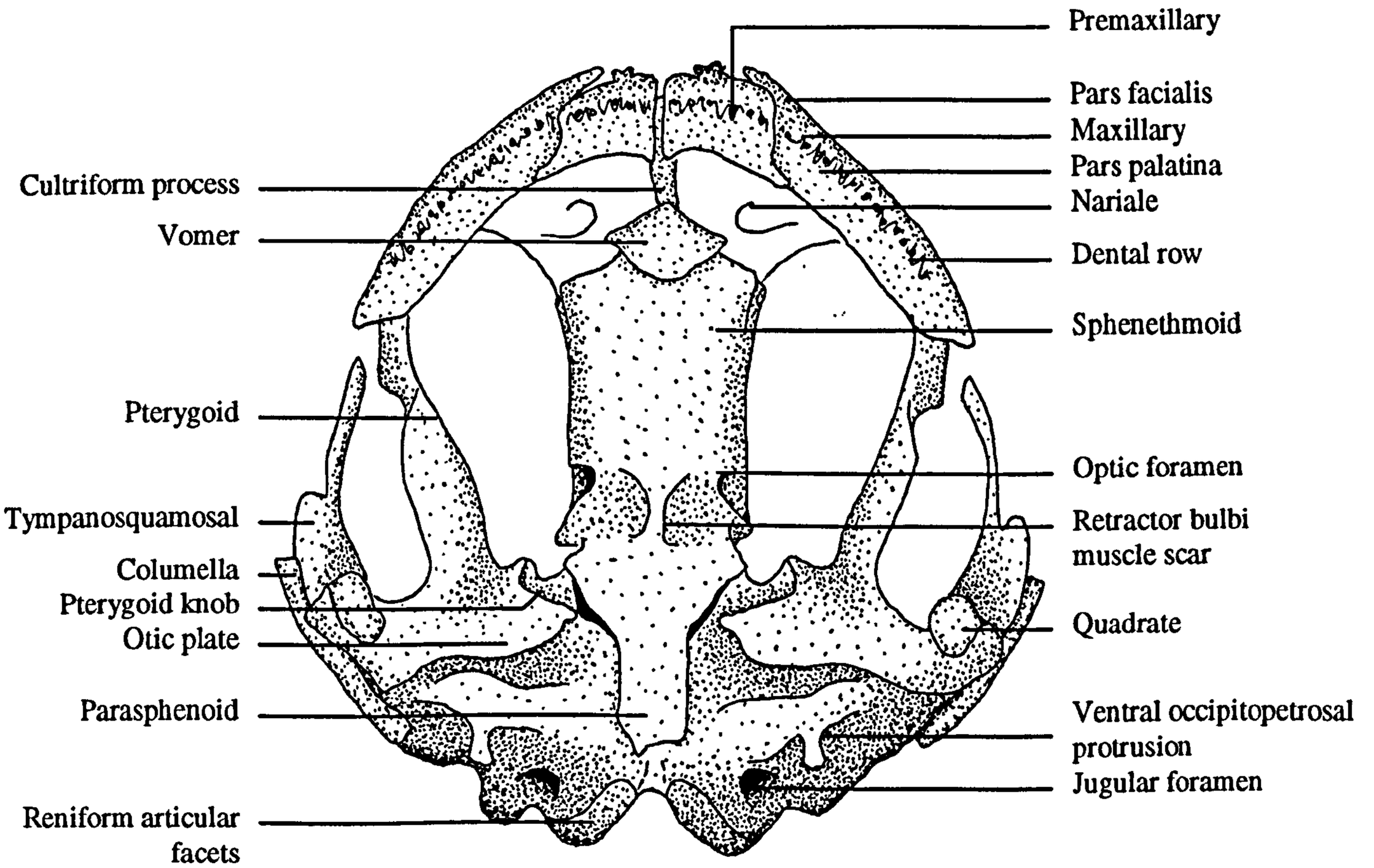
Variation, oddities and deformities

Parasagittal crests may be distinct, forming a dorsal skull table of variable widths, even in specimens which, osteologically, are highly developed. The pineal foramen occurs half way along the element in one specimen. Optic foramina are variable in size. In one specimen, the maxillary arch is unusually long, reaching almost half way across the orbit.



Dorsal view *X. l. victorianus* skull

Illustration 4-6



Ventral view *X. l. victorianus* skull

Illustration 4-7

Axial skeleton

The vertebral column is moderate in length/width (Illustration 4-8). Pre- and postsacral portions of the column are approximately equal in length. The atlas is wide and deep with a straight or convex anterodorsal margin, and narrows towards a posterior margin that bears a single or paired, dorsal spines; all subsequent vertebrae are moderately well-ornamented and bear single, dorsal spines. The first pair of transverse processes is anterolaterally- or perpendicularly-oriented with respect to the vertebral column and is tapered. Each process measures approximately half the width of the atlas. The second pair, measuring approximately twice the length of the first, is oriented posterolaterally. Curvature is minimal and occurs proximally. The third pair, still longer, measure up to 250% the first, are perpendicularly oriented on leaving the column and curve posterolaterally. Curvature occurs along the length of the process. In most specimens, the atlas is highly convex ventrally, with a medially notched anterior margin, and narrows considerably towards the posterior margin. In a minority of specimens however, the atlas bears a straight anteroventral margin, lacks the medial notch and the difference in width between anterior and posterior margins is not so pronounced. Articular faces between centra are moderately narrow and spinal foramen separating the atlas and axis, large. Sacral diapophyses are asymmetrical around the lateral plane in almost all specimens and parallel-sided in the remainder. Vestigial processes are absent from most specimens.

Variation, oddities and deformities

The only 'oddity' recorded in the sample concerns the fourth, left-hand transverse process of a single specimen, which is longer than its corresponding process, and is curved in a similar manner to the second and third transverse process pairs.

Anterior appendicular skeleton

Pectoral girdle

Suprascapular and cleithrum are large, overlapping mid-dorsally (Illustration 4-9). The scapula is short and whilst partes glenoidialis and acromialis are separated by a narrow cleft in most specimens, these may fuse. In the event of fusion, a suture may or may not be present. Clavicles arch anteromedially, their medial margins measuring up to three-times the lateral width of the shaft. The angle formed between their anteromedial margins is acute, and that formed between posteromedial margins obtuse or almost straight. A narrow gap separates clavicles medially. Medial and lateral margins of coracoids are approximately equal in width, though some are wider medially than laterally. The gap separating these elements medially measures between one and one and a half times their medial margins. The degree of calcification of epicoracoid, sternal and suprascapular cartilage is high overall, but variable.

Forelimb

As for description in chapter 2. Phalanges are short.

Posterior appendicular skeleton

Pelvic girdle

Iliac crests extend either in parallel or diverge from the broad U-shaped inter-iliac symphysis. Though synchondrotically united in all specimens, a scar is usually visible between adjacent elements. Components of the pelvic girdle are rarely synostotically fused.

Hindlimb

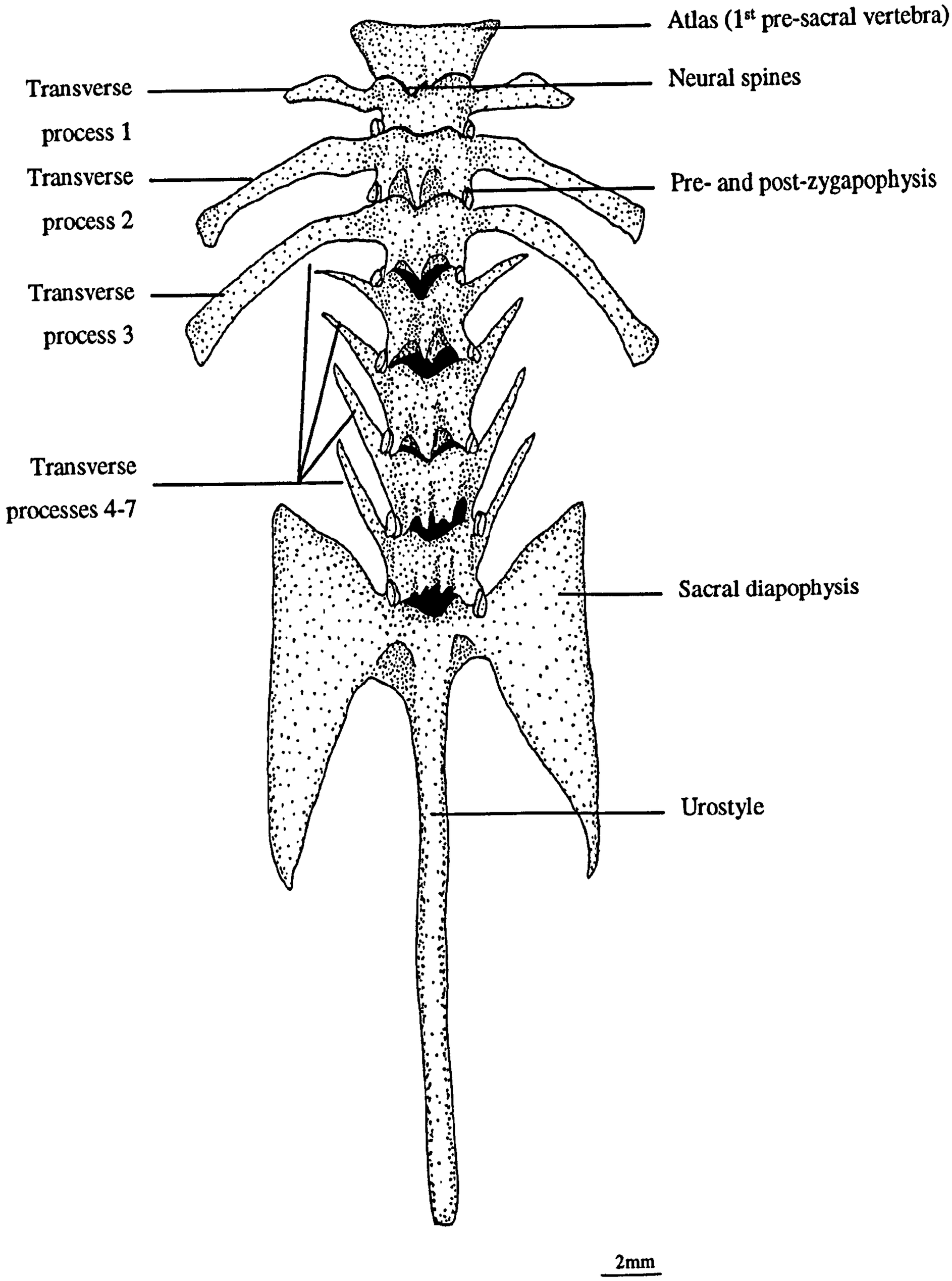
As for account in chapter 2. The oval shaped distal prehallical element measures approximately one and a quarter times the length of the proximal element, also oval.

Sexual Dimorphism

The main form of sexual dimorphism in this taxon besides a difference in snout-vent length of 23% is manifest mainly in differences in the cranium and relative length of transverse processes of the vertebral column. The tympanic annulus is oriented more dorsally in males than in females. Relative skull length, and length of frontoparietal extending anterior from its association with the occipitopetrosal is greater in males than in females, as is the distance between ventral occipitopetrosal protrusions. The length ratio of second-to-third transverse process is greater in males than in females.

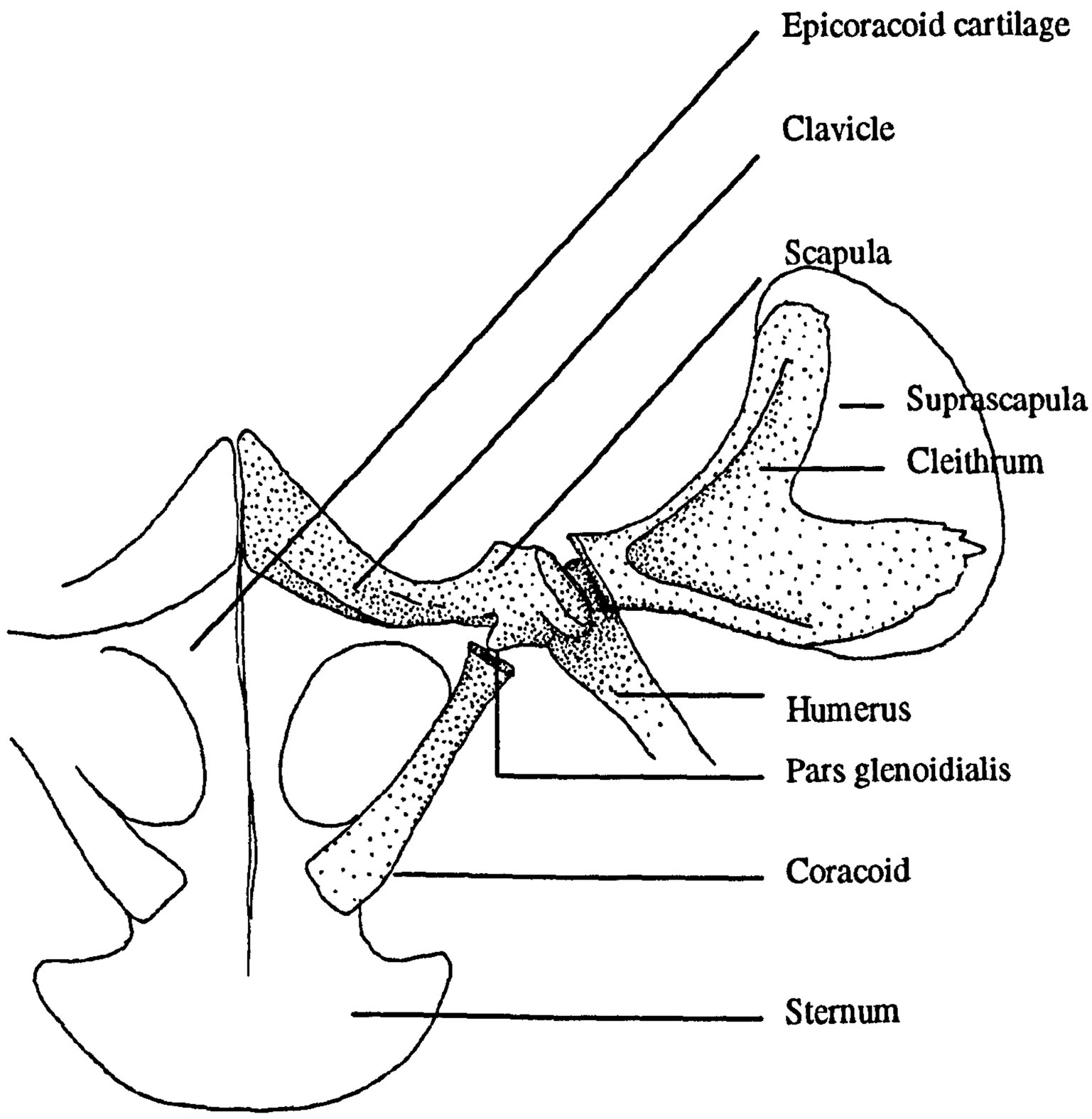
Principal features that distinguish X. l. victorianus *from the remaining three* Xenopus *taxa in this analysis:*

This taxon bears a medially pronounced frontoparietal crest, a narrow inter-rtbs gap, small foramen magnum, well-developed crista parotica, small inter-otic gap, long ventral pterygoid flange, and short anterior pterygoid flange. The taxon lacks posterolateral sphenethmoid alae. The tympanic annulus is short and there is no overlap between zygomatic ramus and anterior pterygoid ramus. The tympanic stalk is well-developed. Optic foramina are small, the vomer narrow, the columella equal in length anterior and posterior to pivot point, the maxillary arch short, the dental row long relative to maxillary arch length and the facial flange narrow. A high degree of calcification characterizes rostral cartilaginous cranial elements, especially the crista parotica. The vertebral column is wide, cleithrum and suprascapular large, scapula short and clavicles medially expanded. The pelvic girdle is characterized by having a broad U-shape inter-iliac symphysis. Prehallux elements are approximately equal in length and the prepollex long.



Dorsal view *X. l. victorianus*
vertebral column

Illustration 4-8



X. l. victorianus pectoral girdle

Illustration 4-9

Xenopus wittei- Osteological Description

Cranium

Frontoparietal

This element is short, broad and parallel-sided, widening slightly at its point of association with occipitopetrosals (Illustration 4-10). It has a rounded caudal profile and an obtusely angled rostral apex. The element lacks antero- and posterolateral alae. The dorsal skull table is variable in shape; parasagittal crests may be widely separated and parallel, diverge in a V-shape, or be sigmoid in shape, widening around the cerebral hemispheres. In some cases, crests diverge towards anterolateral corners of the element, whilst in others they extend anteromedially and do not diverge. In all cases, the posterior margin of the skull table forms a narrow U-shape within the posterior margin of the element. The pineal foramen occurs approximately one-quarter of the way along the element.

Sphenethmoid

This parallel-sided element bears laterally displaced notches along the rostral margin that represent orbitonasal foramina (Illustration 4-11). In some cases, these may be enclosed by bone. Rtbs are widely separated and enclose small optic foramina, the apertures of which are visible in ventral view. Scars extend posterolaterally towards small posterolateral alae, which measure no more than the width of the main sphenethmoid body. Caudally, the element narrows to form a spatulate process that broadens towards its posterior margin and extends almost to the level of the foramen magnum. The vomer varies considerably in both size and shape, and though usually azygous, may also be paired.

Occipitopetrosal

These elements are very deep and narrow (Illustration 4-10, Illustration 4-11). The orientation of anterior margins is sub-perpendicular and posterior margins diagonal, both converging on short cristae parotica. Dorsal crests are long and moderately well developed, occasionally bearing a short, posteromedially directed branch. The remainder of the auditory capsule is convex. Medial margins are almost always fused around the wide foramen magnum. Eustachian troughs are broad with posteromedially directed posterior margins extending towards the widest part of the caudal sphenethmoid process. The posteriorly-directed processes that extend from half way along this ridge are usually short and broad based, but in some specimens are no more than bulges.

Pterygoid

The proximal flange of the anterior ramus is uncleft and the distal flange only moderately curved (Illustration 4-10, Illustration 4-11). The posterior ramus lacks any distinguishing features. Otic plates are asymmetrically bifid; the smaller anterior ramus tapers to a point and extends anteromedially around the pterygoid knob, whilst the larger, medially-directed flange is unevenly

tapered with an anteromedially-oriented posterior margin. The inter-otic gap is wide, measuring approximately the same as the main sphenethmoid body.

Tympanosquamosal

The annulus is round and distinct in its degree of dorsal expansion (Illustration 4-10). The rim is dorsomedially expanded, in most cases obscuring the base of the element at its site of articulation with the crista parotica. The annulus bears a well-developed otic process that supports the columella at its pivot point, and the internal tympanum ala is unusually prominent. There is no overlap between tympanosaquamosal and crista parotica. The squamosal varies in robustness and lacks a distinct zygomatic ramus, extending to articulate with the posterodorsal face of the anteriormost pterygoid flange. Its site of divergence from the annulus is generally marked by a deep cleft. The columella is longer anterior than posterior from its pivot point, is unusually stocky and is expanded rostrally (especially in males).

Maxillary Arch

This long, tapered element reaches half way across the orbit, the dental row extending along the entire length of premaxillaries and along approximately three-quarters the length of maxillaries (Illustration 4-11). The premaxillary is longer than the maxillary relative to those of other taxa. The arch reaches the posterior margin of the anterior-most pterygoid flange. Partes palatina and facialis are moderately broad, giving the appearance of a robust upper jaw.

Nasal and Septomaxillary

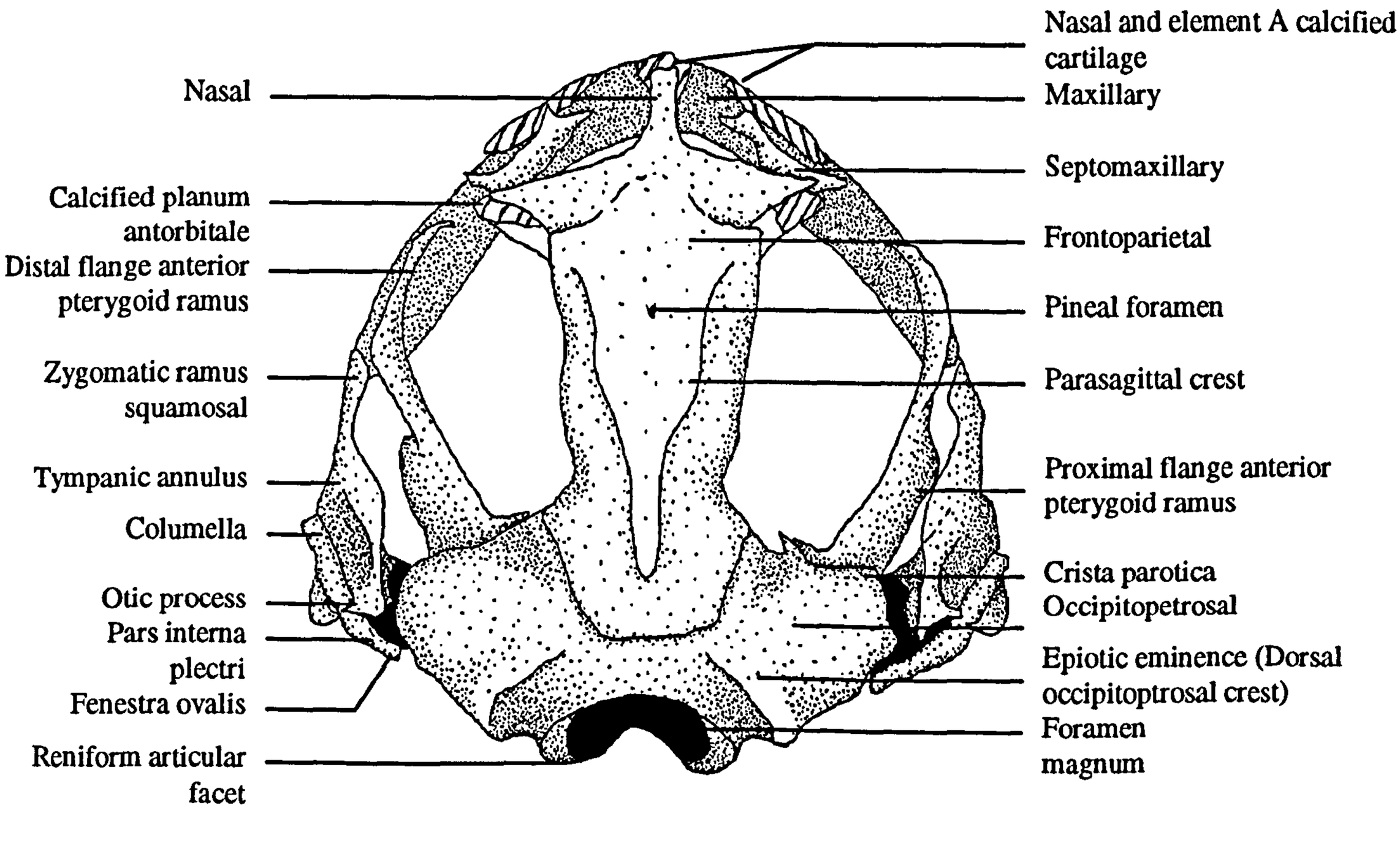
The rostral nasal process is relatively wide, slightly bulbous in most specimens, lacks a medial scar in all but one specimen, and is not cleft (Illustration 4-10). There is a small degree of ventral curvature. Anterolateral margins are sub-perpendicularly oriented with respect to the main body axis, lateral extensions lacking foramen. Lacrimonasal foramina are also absent from septomaxillaries, which are moderate in length and orientation.

Mandible

As for account in chapter 2.

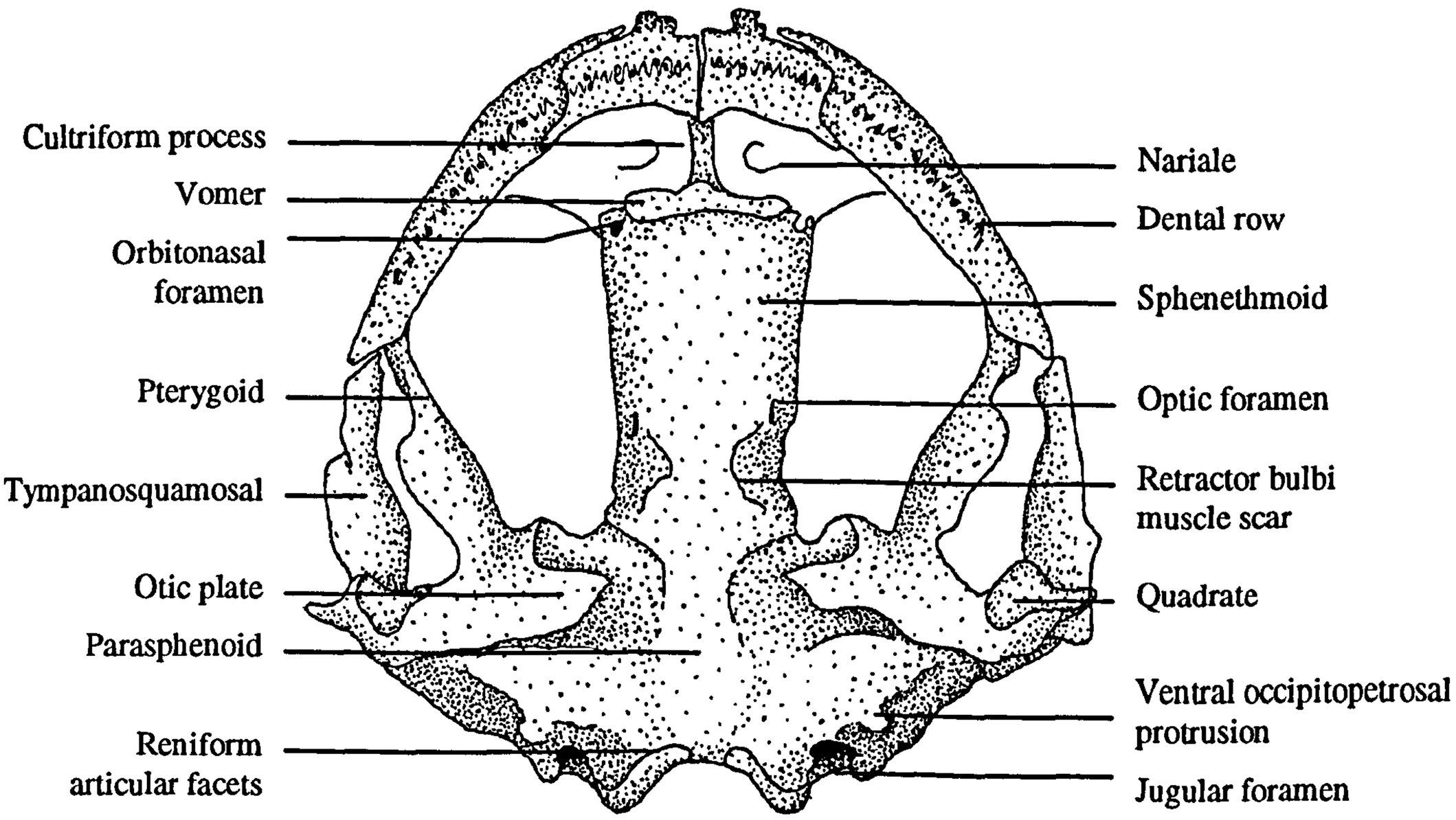
Calcification and fusion of rostral elements

The prevalence and extent of calcification of *all* rostral cartilaginous elements is extreme, higher than in any other species examined towards this thesis, and includes calcification of the planum antorbitale. Fusion of adjacent cranial elements is extensive; sphenethmoid to frontoparietal laterally, frontoparietal to nasal and to occipitopetrosal, sphenethmoid to vomer and occipitopetrosals medially.



Dorsal view *X. wittei* skull

Illustration 4-10



Ventral view *X. wittei* skull

Illustration 4-11

Axial skeleton

The column is long and narrow, as determined by relative width of vertebrae, transverse processes and sacral diapophyses. The pre-sacral column length is greater than post-sacral length. Dorsally the atlas is deep with a convex anterior margin. The posterior atlas margin is slightly narrower and bears widely separated, paired dorsal spines. Subsequent vertebrae are not highly ornamented and bear single spines. Zygapophyses may occur just within or on the outer margin of the column. The first pair of transverse processes is oriented anterolaterally or perpendicularly with respect to the main body axis. Processes are either parallel or are widest half way along their length, and tapered distally. They measure approximately the same in length as the atlas width. The second pair measures between 130 and 200% of the first, is anterolaterally oriented on leaving the vertebral column, curving posterolaterally; curvature is mainly proximal. The third pair is equal in length to the second, though curvature and the degree of distal expansion are greater. Ventrally, the atlas bears a highly convex, medially notched anterior margin. Articular faces between centra are narrow, and spinal foramen between adjacent pedicels small. Sacral diapophyses are asymmetrical around the lateral plane, parallel-sided and, in all except one specimen, bear no vestigial processes.

Variation, Oddities and Deformities.

Paired vestigial processes occur in one specimen are partially fused along their distal margins to the posterior margin of the sacrum, exposing large foramina where they remain unfused.

Anterior appendicular skeleton

Pectoral girdle

Suprascapula and cleithrum are moderate in size (larger than *X. fraseri,* smaller than *X. laevis)* and almost touch mid-dorsally. The scapula is moderately long, and in most, partes glenoidialis and acromialis are indistinguishably fused, although some bear scars and a few remain unfused. Clavicles arch anteromedially and are medially expanded, their medial widths measuring approximately twice the width of the shaft, proximally. An acute angle is formed between anterior margins and an obtuse angle between posterior margins.

The coracoid is only minimally waisted, its lateral and medial margins approximately equal in width. Medial margins are usually separated by a gap that measures approximately twice (though some as little as x0.2 times) the medial width of the element. The extent of calcification of epicoracoid, suprascapular and sternal cartilage is high.

Forelimb

As for account in chapter 2. Phalanges are moderately short.

Posterior appendicular skeleton

Pelvic girdle

Iliac processes extend in parallel or diverge from the broad U-shape inter-iliac symphysis.

Hindlimb

As for account in chapter 2. Proximal prehallical elements are oval in shape and measure approximately half the length of distal elements, which are triangular in shape and flat in cross section.

Sexual dimorphism

Males differ from females primarily in being smaller. Femur and fibio-tibula are longer in males than in females however. The braincase, in the region of auditory capsules, is larger and the pineal foramen positioned further anteriorly in males than females. The structure of the tympanic annulus also demonstrates a high degree of sexual dimorphism. In males, the pars externa plectra is grossly enlarged, obscuring the entire crista parotica. The apparatus is more dorsally-oriented than in females, and extends anteriorly almost to the level of the zygomatic ramus.

Principal features that distinguish X. wittei *from other taxa in this analysis*

The frontoparietal is parallel-sided with diverging parasagittal crests and rostrally-positioned pineal foramen. The rtbs are widely spaced, the inter-otic plate gap and foramen magnum wide. The zygomatic ramus articulates with the posterodorsal face of the pterygoid anterior ramus. The maxillary is long and tapered. Cristae parotica are short, and associate with well-developed posterolateral processes on the tympanosquamosal. The dorsally expanded tympanic annulus sometimes overhangs the base of the element. The tympanic annulus is long, bearing prominent internal alae. The suprascapula and cleithrum are moderate in size. Most clavicles are partially expanded, medially. The columella is stocky and wide anteriorly. Fusion between adjacent cranial elements and the degree of calcification of rostral cranial elements extensive.

Xenopus vestitus- Osteological Description

Cranium

The skull is long and narrow.

Frontoparietal

The frontoparietal is usually straight-sided, and slightly narrower anteriorly than at its anterior-most point of association with occipitopetrosals (Illustration 4-12). Anterolateral margins are rounded, and converge on an obtusely angled apex. The caudal profile is U-shaped, narrowing towards its base and extending almost to the level of the foramen magnum. In some specimens small, posterolateral alae are present and are separated by a narrow gap. Parasagittal crests form a prominent, medial ridge that extends from inside the posterior margin of the element to the level of the pineal foramen. Here, crests may diverge slightly, but do not reach anterolateral margins of the element. In no specimen is there any sign of a dorsal skull table, or of medial crest migration with growth. Brain case walls, lateral to this crest are flat, except in the region of the cerebral hemispheres where they are convex. The pineal foramen is positioned approximately a quarter of the way along the element from the apex.

Sphenethmoid and vomer

Lateral margins of this element are straight (Illustration 4-13). They are usually parallel, occasionally converging to a small degree towards the rostral margin. Positioned laterally along this margin in some specimens are paired notches, representing orbitonasal foramina. Rtbs are separated medially by a narrow gap and enclose small, laterally-displaced optic foramina, the apertures of which are visible only in lateral view. (In smaller specimens, optic foramina are larger and assume a more ventral position.) Caudal margins of rtbs extend towards prominent posterolateral alae that are contiguous in some specimens with a medially-directed bony ridge. The caudal sphenethmoid process widens towards its posterior margin, but does not reach as far as the foramen magnum. The vomer is large and edentulous, and may be either paired, azygous or multiply fractured; vomers, in 8/19 cases are paired, in 7/19 are azygous and in the remaining 4/19 are multiply fractured. In 8/19 specimens, the vomer is at least partially fused to the sphenethmoid.

Occipitopetrosal

This element is relatively narrow, with a perpendicular anterior and obliquely oriented posterior margin that converge laterally on short crista parotica (Illustration 4-12, Illustration 4-13). Medial margins vary between being being unfused to fully fused around the wide foramen magnum, which, forms a deep, inverted V-shape dorsal margin.

Dorsal crests are long and highly developed, sometimes even forming an ossified overhang; the angle formed with the main body axis is relatively high. Both length and the angle formed reflecting the narrowness of the caudal frontoparietal margin and relative breadth of the foramen magnum. The dorsal surface of auditory capsules is flat. Ventrally, Eustachian troughs circumvent auditory capsules, and account for approximately half the depth of the element. The bony ridge that borders the trough extends posteromedially and bears a particularly prominent, posteriorly directed process.

Pterygoid

Proximal flanges of the anterior pterygoid ramus are slightly convex, rather than straight, with respect to one another, and the gap separating their posteromedial margins narrow relative to that of other taxa (Illustration 4-13). The anterior flange is short and narrow and the degree of distal curvature high. The lateral ramus lacks any distinguishing features. Otic plates are only barely bifid. They are separated medially by an unusually large inter-otic gap. The anterior region of the plate rests on the pterygoid knob.

Tympanosquamosal

The round tympanic annulus is very small with posterodorsal and ventral processes that support the columella at its pivot point (Illustration 4-12). A particularly long, slender squamosal extends from the anterodorsal margin of the annulus to overlap and articulate with the dorsal face of the anterior pterygoid flange by means of a highly expanded zygomatic ramus (occasionally, specimens may possess a larger annulus, and correspondingly shorter squamosal). The junction formed between squamosal and tympanic annulus is broad and U-shaped. At its *(well-developed)* stalk-like base, the tympanosquamosal partially overlaps the occipitopetrosal. The columella is longer anterior than posterior from its pivot point with the annulus.

Maxillary Arch

Distally, maxillaries are highly tapered, extending approximately half way across the orbit (Illustration 4-13). The dental row extends the length of premaxillaries and along two-thirds the length of maxillaries, and the arch reaches beyond the posterior margin of the anterior pterygoid flange. The premaxillary is relatively short. Partes palatina and facialis are robust and alary processes are almost always bifid, but are occasionally spatulate.

Nasal and Septomaxillary

The rostral nasal extension is usually parallel-sided, is moderately wide and lacks a medial scar. The element is distally uncleft and exhibits a degree of ventral curvature. Lateral extensions lack lacrimonasal foramina and their anterior margins are sub-perpendicular in orientation. Septomaxillaries are relatively long and oriented at a low angle with respect to the main body axis.

The lateral part of this element, in some specimens, is pierced by a large lacrimonasal foramen, through which sub-ocular tentacles pass.

Mandible

As for account in chapter 2 (Illustration 4-14).

Calcification

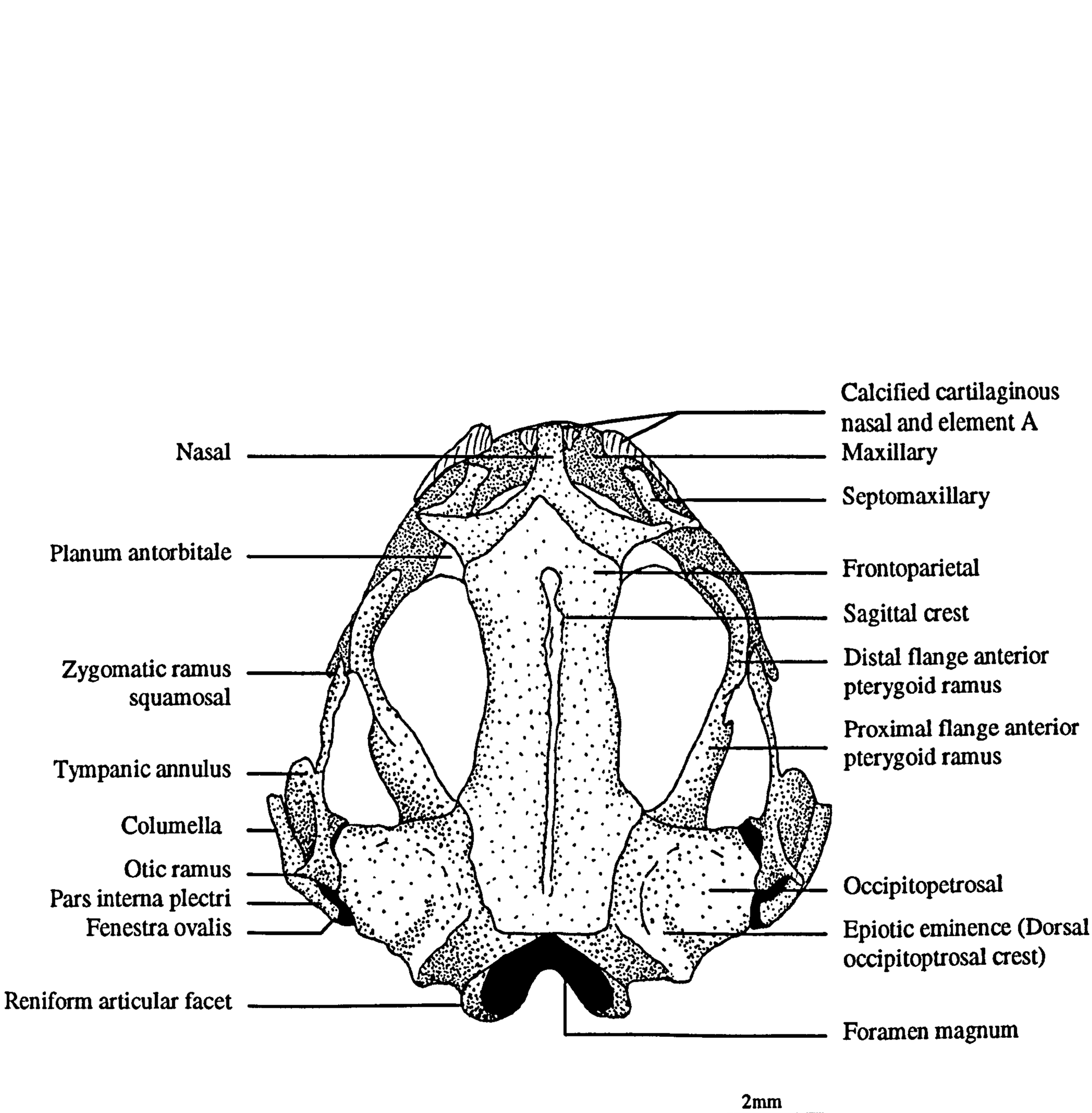
The prevalence of calcification of rostral elements is remarkably high, as is the extent to which elements are calcified (nasal floating, alary, septum nasi, and nasal flanking) but interestingly, hardly ever includes the planum antorbitale.

Fusion

The degree of fusion between adjacent cranial elements is mainly restricted to fusion between sphenethmoid and occipitopetrosal, though occasionally, the frontoparietal may fuse to sphenethmoid laterally, nasals or the occipitopetrosal dorsally.

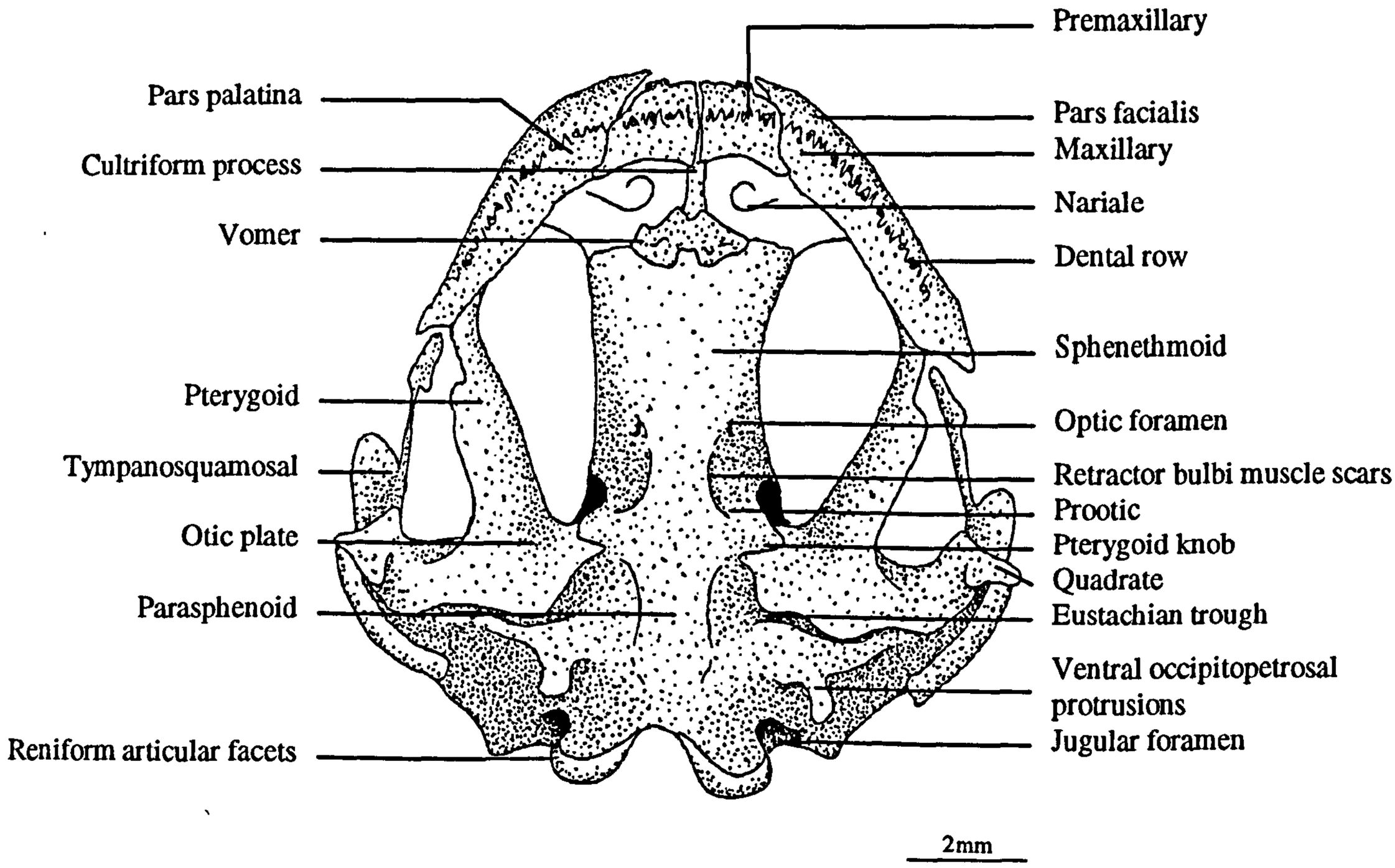
Variation, oddities and deformities

In small specimens, parasagittal crests are barely distinguishable, but always appear to be in medial association.



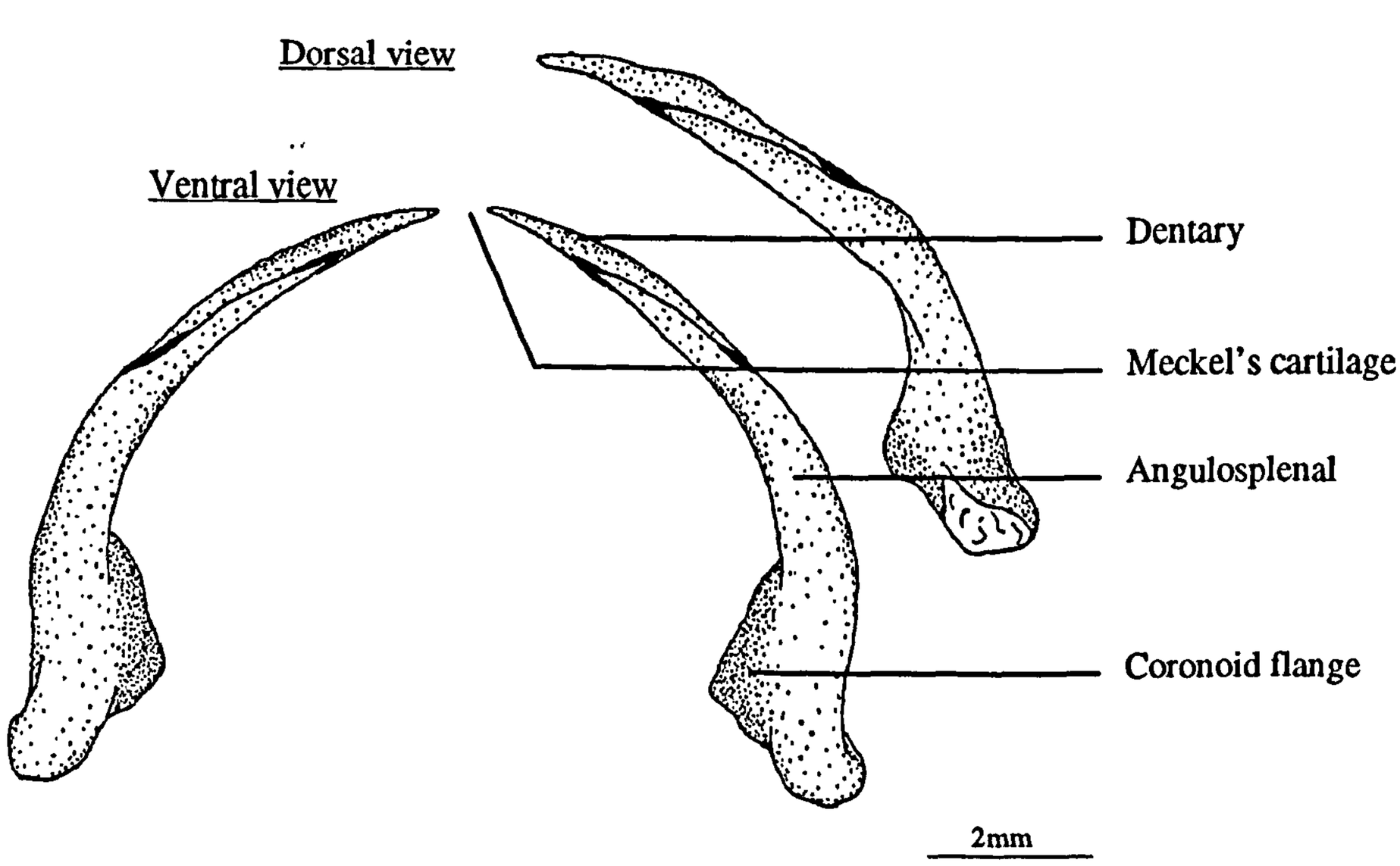
Dorsal view *X. vestitus* skull

Illustration 4-12



Ventral view *X. vestitus* skull

Illustration 4-13



X. vestitus mandible

Illustration 4-14

Axial Skeleton

The vertebral column is long and narrow, as determined by widths of transverse processes and sacral diapophyses, relative to column length (Illustration 4-15, Illustration 4-16). The pre-sacral portion of the column is considerably longer than the post-sacral portion. The atlas is moderately concave anterodorsally, and narrows towards its caudal margin, which usually bears paired dorsal spines. All subsequent vertebrae are moderately ornamented and bear single dorsal spines. The first pair of transverse processes is oriented anterolaterally on leaving the vertebral column; they are short, measuring approximately half the maximum width of the atlas, widest mid way along their length, and taper distally. The second pair measure approximately 150% of the first, is perpendicular on leaving the column, curve posterolaterally, and are moderately expanded distally. Curvature occurs mainly half way along the element. The third pair is slightly shorter than the second; curvature is greater and either occurs evenly along the length of processes or mainly mid-way along the process, as for the second pair. Ventrally, the anterior atlas margin is convex and medially notched. Articular faces between adjacent centra are narrow, and the spinal foramen separating pedicels large. Sacral diapophyses are asymmetrical around the lateral plane in the majority of cases and have parallel sides. Vestigial processes are absent in all except for a single specimen.

Variation, oddities and deformities

The prevalence of fusion between atlas and axis in this sample is high, at 48%. In many of these however, fusion is only partial, and in every case, a scar marks the site of fusion between elements. The length of this compound vertebra is approximately equivalent to the sum of the two- i.e. they are not condensed into one, as is the case in *Silurana* and *X. largeni.* In a single specimen, transverse processes 4-8 include some deformed processes. These are either branched or resemble the state seen in the second and third pair of transverse processes. The sacrum is also deformed in one specimen, and bears a transverse scar and foramen as if representing partial fusion between two elements.

Anterior appendicular skeleton

Pectoral girdle

Cleithrum and suprascapula are small, and do not meet mid-dorsally. Partes glenoidialis and acromialis of the long scapula are cleft. Clavicles arch anteromedially, their medial margins measuring approximately three times the lateral width of the shaft; the angle formed between anterior margins is acute, and between posterior margins almost straight. Coracoids are as wide medially as they are laterally and are separated medially by a gap that measures between one and one and a half times the lateral width of the element. Calcification of suprascapula, epicoracoid and sternal cartilages occurs to varying degrees.

Forelimb

As for account in chapter 2, with the general exception that the distal end of humerus is relatively wide, compared with that of other taxa examined (except for *X. largeni*). Phalanges are particularly short.

Variation, deformities and oddities

One specimen demonstrated an unusual deformity, and one that warrants special attention; the appearance of an additional carpal element between and distal to carpal elements I+II, which is 'tethered' by a separate muscle, and as such is not a shard of an adjacent fractured element.

Posterior appendicular skeleton

Pelvic girdle

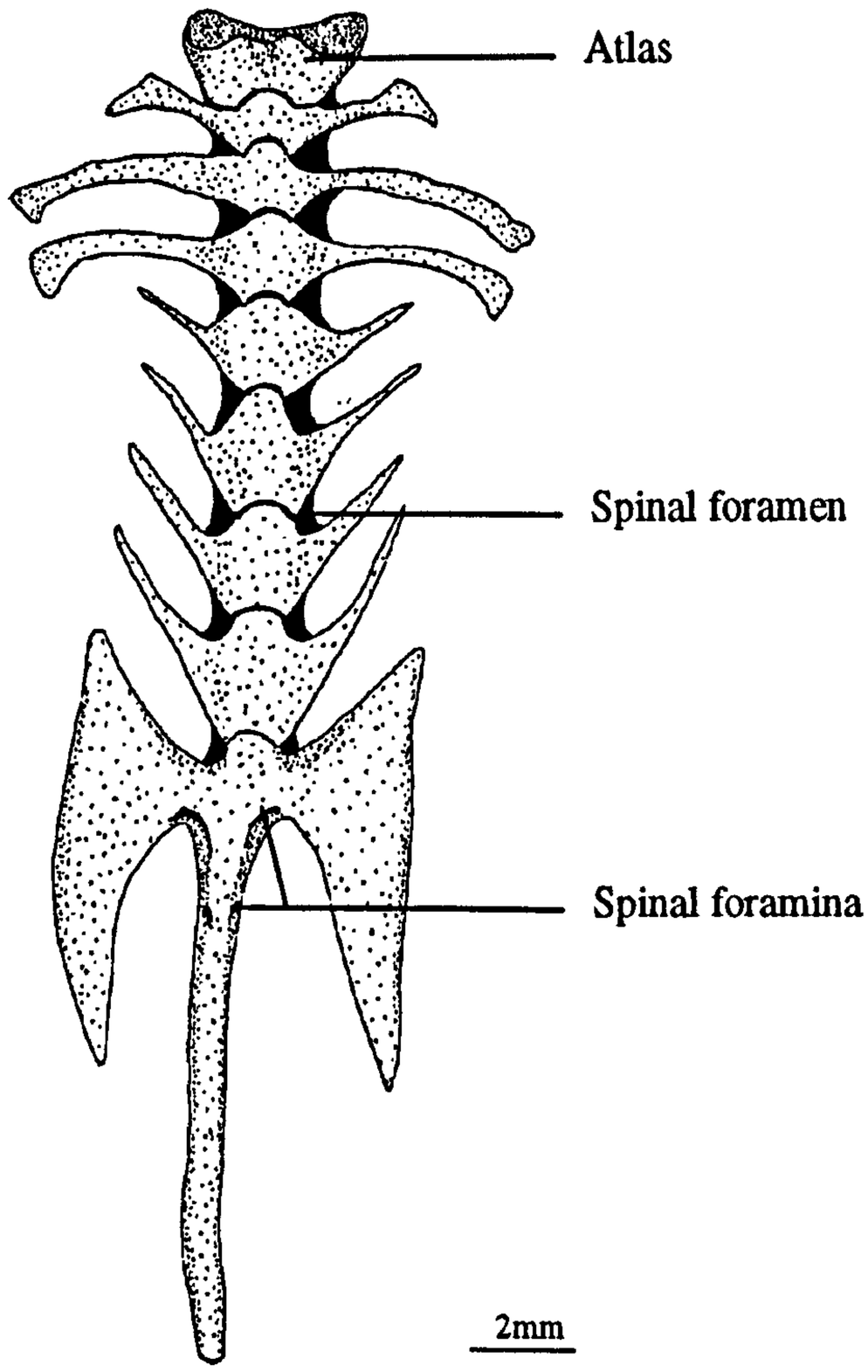
Iliac processes diverge from a particularly narrow V-shaped inter-iliac symphysis, which is flanked by unusually large dorsal prominences. The degree of fusion between elements is variable, and in specimens that exhibit an advanced state of ossification, a scar remains visible between all adjacent elements.

Hindlimb

As for account in chapter 2. The distal prehallical element is slightly longer than the proximal of the pair, and is tapered/oval in shape. The proximal element is oval and phalanges are short.

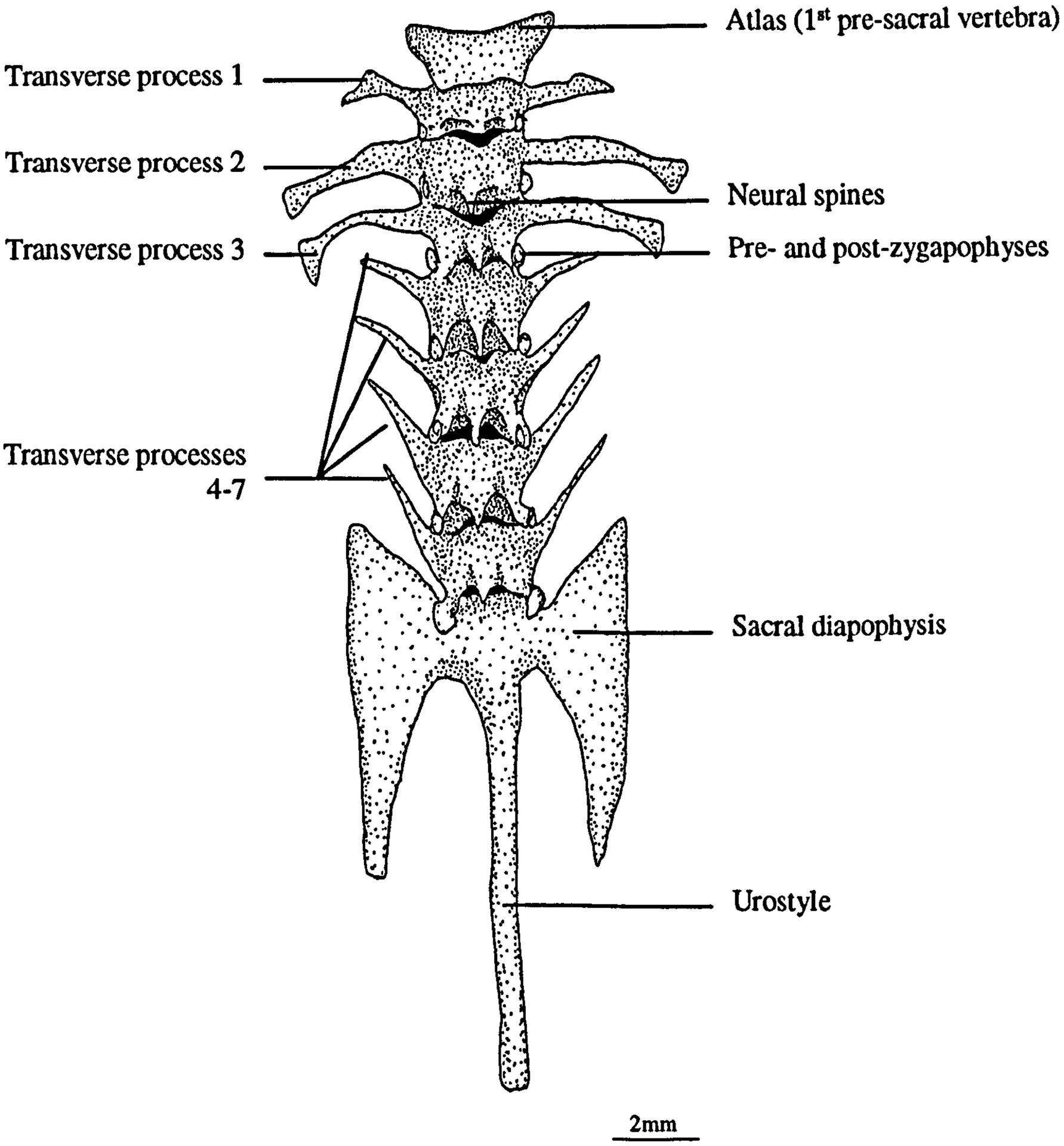
Sexual dimorphism

Males are on average 12.5% shorter than females. The skull is narrower and longer in males, but bears a wider foramen magnum and nasal. Despite the orientation of tympanic annuli being indistinguishable between sexes (unusually), the annulus length is greater in males than in females.



Ventral view *X. vestitus* vertebral column

Illustration 4-15



Dorsal view *X. vestitus* vertebral column

Illustration 4-16

Principal features that distinguish X. vestitus *from other taxa in this analysis*

The skull is long and narrow, being more pointed than rounded in anterior profile. The frontoparietal is parallel-sided with with more of a V-shaped posterior margin than in other taxa. Parasagittal crests unite to form medially raised ridge that extends to posterior margin of element and diverges only slightly at level of pineal foramen. A small gap separates rtbs, which extend to form distinct posterolateral sphenethmoid alae. The caudal sphenethmoid process widens towards its posterior margin. Oblique anterior and perpendicular posterior margins characterize occipitopetrosals, which have short cristae parotica, an inverted V-shape dorsal foramen magnum margin and highly developed dorsal crests which are oriented at low angle with respect to main body axis. Otic plates are barely bifid, separated by a very wide inter-otic plate gap. A relatively short gap separates bases of anterior pterygoid rami, these elements having a short distal flange. The tympanic annulus is short, bearing a long squamosal arm and broadly expanded zygomatic ramus, which articulates dorsally with pterygoid. There is a degree of dorsal overlap between the tympanic annulus stalk and occipitopetrosal. Posterodorsal and ventral processes of the tympanosquamosal support the columella. The distally tapered maxillary arch reaches half way across orbit, its dental row extending along two-thirds the length of maxillary. Septomaxillaries are pierced by large foramen. A very high degree of calcification of rostral elements, except for planum antorbitale, characterizes the species. The vertebral column is long and narrow, with a longer pre- than post-sacral portion. The atlas bears paired dorsal spines whilst other pre-sacral vertebrae bear single spines. Transverse processes are short. The cleithrum and suprascapular are small, and the medial clavicle margins medially expanded. The humerus is robustly proportioned. Phalanges are short, the pelvic girdle narrow and distal prehallical element semi-conical, on average measuring 1.3 times the proximal element.

DISCUSSION

Snout-vent length for samples of all species was considerably smaller than those reported in the literature (see Kobel *et al.*, 1996). The degree of sexual dimorphism observed in snout-vent length was equivalent to that recorded in the literature for *X. l. victorianus*, whilst being less than that previously recorded for *X. wittei* and *X. vestitus* and greater than recorded for *X. fraseri aff.*. The magnitude of this variation was inter-specifically variable. There was also a high degree of inter-specific variability in the form taken by sexual dimorphism, a different combination of characters accounting for most of the sex-related variation in each species (Table 4-3). Distinguishing features for each species have been incorporated into individual species accounts.

Results from univariate and multivariate analyses conducted to identify the most sexually dimorphic characters were largely congruent. Variation between these sets of results may have been a result of disparity introduced by differential methods of size-standardization in each of the two forms of analyses. Correlation between variables may also have affected results in multivariate analyses (Willig, Owen and Colbert, 1986; Corruccini, 1987). Nevertheless, these results combine to give an overall impression of the principal form of sexual dimorphism and how this differed inter-specifically.

Variation between each species when compared with the other three (combined and individually) was shown to be highly significant. According to DFA, the most discriminatory characters (listed in Table 4-5 and Table 4-6) were found to be highly concordant between pooled-sex and separate-sex analyses, except in the case of *X. l. victorianus*, indicating that the characters accounting for most of the inter-specific variation are different from those accounting for sexual dimorphism in all except for *X. l. victorianus*. Anomalous results for this taxon were explained in terms of characters accounting for most of the inter-specific variation also demonstrating a degree of sexual dimorphism.

Phenetic similarity

The pattern of phenetic similarity between taxa (Figure 4-3) indicates that *X. wittei* is almost perfectly intermediate between tetraploids, *X. fraseri* and *X. l. victorianus*. *Xenopus vestitus*, in contrast, is as distinct from each tetraploid as they are from one another. This provides strong evidence for the involvement of both *X. fraseri aff.* and *X. l. victorianus* in the hybrid origin of both *X. wittei* and *X. vestitus*. The pattern of clustering shown in Figure 4-3 suggests that an extended period of divergence is likely to have passed since the *X. vestitus* hybridization event, compared to that of *X. wittei*. An alternative explanation could be that *X. vestitus* has undergone extreme genetic

bottlenecking, which has resulted in an accelerated rate of propagation of aberrant genes throughout the population and of more marked divergence from the remainder of the genus.

Both interpretations are consistent with evidence from studies comparing globin peptides between species (Burki and Fischberg, 1985); the pattern of band migration demonstrated by *X. wittei* was highly complex compared with that of *X. vestitus*, suggesting more extensive silencing of globin genes in the latter species. The functional silencing of duplicate loci is characteristic of advanced diploidization (Ferris and Whitt, 1977), and implies a more ancient origin for *X. vestitus*. Divergence time is recognized by Kobel and DuPasquier (1986) as being an important factor in the process of diploidization. This is evidenced in studies on the patterns of globin migration by Jeffreys, Wilson, Wood and Simons (1980) and Burki, Shwager and Fischberg (1984). Vonwyl and Fischberg (1980) have demonstrated a similar phenomenon, namely, the appearance of additional LDH isozyme sub-units in higher ploidy *Xenopus* species. Further evidence supporting an advanced state of diploidization in *X. vestitus* as compared with *X. wittei* is provided by experimental hybridization of each with a sibling *X. laevis* sub-species. In the case of *X. wittei*, eggs are fertilizable by sperm of *X. l. laevis*, whilst the eggs of *X. vestitus* demonstrate a fertilization block to *X. l. laevis* sperm (Tinsley *et al.*, 1979).

Each of these evolutionary processes, genetic bottlenecking and a prolonged period of genetic isolation, is plausible given what we now know of recent environmental changes. Indeed, each may have affected the population during the period since it arose. If evidence of advanced diploidization in *X. vestitus* is accepted as are indications that the hybridization events leading to the evolution of each of these taxa arose during the Pleistocene, it is possible that a period of up to 2.5 million years separates the origin of each of these octoploids, *X. vestitus* having arisen earlier than *X. wittei*. It is possible that *X. vestitus* has experienced up to 21 more periods of significant habitat disturbance than *X. wittei* (see Tinsley, 1996), each placing the species under extreme pressure. The phenetic distance seen to separate octoploids, *X. vestitus* and *X. wittei*, provides support for this.

An alternative interpretation of these results would be that an extinct or as yet undiscovered tetraploid form was involved in the hybridization event that gave rise to *X. vestitus*. Whilst possible, no supporting evidence has yet come to light for this. Should such evidence be discovered, the osteological description provided by the current study would provide a means of confirming its taxonomic status as one of the potential parent taxa of *X. vestitus*.

CONCLUSION

The distribution ranges of *X. fraseri* and *X. l. victorianus* are known to overlap in a number of sites in Central Africa (Laurent, 1972), and probably did so with greater frequency during interglacial periods of the Pleistocene (Tinsley *et al.*, 1996). This will have repeatedly provided the physical

opportunity for hybridization, and is consistent with evidence from a range of published sources of their involvement in the hybridization events leading to the production of octoploid taxa, *X. wittei* and *X. vestitus*. Whilst inference drawn from shared osteological affinities allies *X. wittei* with each tetraploid, relations for *X. vestitus* remain ambiguous. Although it is possible that *X. vestitus* arose from a hybridization event between *X. fraseri aff.* and *X. l. victorianus*, the taxon has diverged to such an extent as to obscure evidence of a direct relationship. Differences in the relative degree of similarity between each octoploid and their postulated parent taxa can however be interpreted in terms of the possibility of there having been a differential in the evolutionary history of each octoploid.

In order to overcome ambiguities that continue to surround the interpretation of relatedness of *X. vestitus*, further comparative analyses are needed to determine relations between species associated, past and present, with this highland complex.

BIBLIOGRAPHY

Ahl, E. (1926). "*Xenopus stromeri* Ahl n. sp." In die Diamantenwuste Sudwest-Africas 2: 141-142. (Ed. Kaiser, E.) Verlag von Dietrich Reimer (Ernst Vohnsen), Berlin.

Bonnefille, R., Riollet, G., Buchet, G., Icole, M., Lafont, R. and Arnold, M. (1995). Glacial/ Interglacial record from intertropical Africa, high resolution pollen and carbon data at Rusaka, Burundi. *Quarterly Science Review* **14(9)**:917-936.

Boulenger, G.A. (1905). On a collection of batrachians and reptiles made in South Africa by C.H.B Grant, and presented to the British Museum by Mr. C.D. Rudd. *Proceedings of the Zoological Society, London* (**2**): 248-255.

Burki, E. and Fischberg, M. (1985). Evolution of globin expression in the genus *Xenopus* (Anura: Pipidae). *Molecular Biology and Evolution* **2**:270-277.

Burki, E., Schwager, J. and Fischberg, M. (1984). Electrophoretic patterns of hemoglobin in different *Xenopus* species, subspecies and interspecies hybrids. *Experimentia* **40**:59-64.

Carcasson, R.H. (1964). A preliminary survey of the zoogeography of African butterflies. *East African Wildlife Journal* **2**:122-157.

Carr, S.M., Brothers, A.J. and Wilson, A.C. (1987). Evolutionary inferences from restriction maps of mitochondrial DNA from nine taxa of *Xenopus* frogs. *Evolution* **41**: 176-188.

Corruccini, R.S. (1987). Univariate versus multivariate morphometric variation in *Eptesicus fuscus*. *Journal of Mammology* **64**:437-444.

de Klerk, H.M., Crowe, T.M., Fjeldsa, J. and Burgess, N.D. (2002). Patterns of species richness and narrow endemism of terrestrial bird species in the Afrotropical region. *Journal of Zoology, London* **256**:327-342.

De Jong, M. and Congdon, C. 1993. "The montane butterflies of the eastern Afrotropics". Pp. 133-172 in Biogeography and ecology of the forests of eastern Africa. Cambridge University press.

Ferris, S.D and Whitt, G.S. (1977). Loss of duplicate gene expression after polyploidisation. *Nature (London)* **265**:258-260.

Fjeldsa, J. (1994). Geographical patterns for relict and young species of birds in Africa and South America and implications for conservation priorities. *Biodiversity and Conservation* **3**:207-226.

Fjeldsa, J., Ehrlich, D., Lambin, E. and Pins, E. (1997). Are biodiversity 'Hotspots' correlated with current ecoclimatic stability? A pilot Study Using the NOAA-AVHRR Remote Sensing Data. *Biodiversity and Conservation* **6**:401-422.

Fjeldsa, J. and Lovett, J.C. (1997). Biodiversity and environmental stability. *Biodiversity and Conservation* **6**:315-323.

Gollmann, G. (1991). Osteological variation in *Geocrinia laevis*, *Geocrinia victoriana*, and their hybrid populations (Amphibia, Anura, Myobatrachinae). *Zeitschrift fuer zollogische systematic und evolutionsforschung* **29**:289-303.

Graf, J.-D. 1996. "Molecular approaches to the phylogeny of *Xenopus*". Pp. 379-388 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Graf, J.-D. and Fischberg, M. (1986). Albumin evolution in polyploid species of the genus *Xenopus*. *Biochemical Genetics* **24**: 821-837.

Hamilton, A.C. ed. 1982. Environmental History of Africa: A Study of the Quaternary. London: Academic Press.

Hamilton, A. (1976). The significance of patterns of distribution shown by forest plants and animals in tropical Africa for the reconstruction of upper Pleistocene Palaeoenvironments: A Review. *Palaeoecology of Africa* **9**:63-97.

Harper, D.G.C. (1994). Some comments on the repeatability of measurements. *Ringing and Migration* **15**:84-90.

Howell, K.M. 1993. "Herpetofauna of the Eastern African forests". Pp. 173-201 in Biogeography and ecology of the forests of eastern Africa. Cambridge University press.

Jackson, J.A., Tinsley, R.C. and Kigoolo, S. (1998). Polyploidy and parasitic infection in *Xenopus* species from western Uganda. *Herpetological Journal* **8 (1)**:16-22.

Jeffreys, A, Wilson V., Wood, D. and Simmons, J.P. (1980). Linkage of adult α- and β-globin genes in *X. laevis* and gene duplication by tetraploidization. *Cell* **21**:555-564.

Jolly, D., Taylor, D., Marchant, R., Hamilton, A., Bonnefille, R., Buchet, G. and Riollet, G. (1997). Vegetation dynamics in central Africa since 18,000 yr BP: pollen records from the interlacustrine highlands of Burundi, Rwanda and western Uganda. *Journal of Biogeography* **24(4)**:495-512.

Kobel, H.R., Du Pasquier, L. and Tinsley, R.C. (1981). Natural hybridization and gene introgression between *Xenopus gilli* and *Xenopus laevis laevis* (Anura: Pipidae). *Journal of Zoology, London* **194**:317-322.

Kobel, H.R., Loumont, C. and Tinsley, R.C. (1996). "The extant species." Pp. 9-33 in The biology of *Xenopus*, edited by Tinsley, R.C., and Kobel, H.R. Oxford: Clarendon Press.

Kobel, H.R., Barandun, B. and Thiébaud, ChH. (1998). Mitochondrial rDNA phylogeny in *Xenopus*. *Herpetological Journal* **8 (1)**:13-18.

Kobel, H.R. and Du Pasquier, L. (1986). Genetics of polyploid *Xenopus*. *Trends in Genetics* **2**:310-315.

Kobel, H.R. and Muller, W.P. (1981). Zytogenetische Verwandtschaft zwischen zwei tetraploiden *Xenopus*. *Arten. Arch. Genet.* **49/50**:188.

Laurent, R.F. (1972). Amphibiens. Exploration Parc national des Virungas. II. **Serial 22**:1-125.

Loumont, C. (1986). *Xenopus pygmaeus*, a new diploid pipid frog from rain forest of equatorial Africa. *Revue Suisse Zoologie* **93**:755-764.

Lovett, J.C. 1993. "Climatic history and forest distribution in eastern Africa." Pp. 23-29 in Biogeography and Ecology of the Rainforests of Eastern Africa, edited by Wasser, S.K. and Lovett, J.C. Cambridge: Cambridge University Press.

Loveridge, A. (1932). New races of skink (*Siaphos*) and frog (*Xenopus*) from the Uganda Protectorate. *Proceedings of the Biology Society of Washington* **45**:113-115.

Marchant, R., Taylor, D. and Hamilton, A. (1997). Late Pleistocene and Holocene history at Mubwindi swamp, southwest Uganda. *Quaternary Research* **47(3)**:316-328.

Moreau, R.E. (1962). Vicissitudes of the African Biomes in the late Pleistocene. *Proceedings of the Zoological Society, London* **121**:395-421.

Reumer, W.F. (1985). Some aspects of the cranial osteology and phylogeny of *Xenopus* (Anura, Pipidae). *Revue Suisse Zoologie* **92** (4):969-980.

Ryan, B.F., Joiner, B.L., Ryan, T.A. (1985). *MINITAB Handbook* (second edition). Duxbury Press: New York, NY.

Tattersfield, P. (1998). Patterns of diversity and endemism in East African land snails, and the implications for conservation. *Journal of Conchology* **S2**:77-86.

Thiebaud, C.H., and Fischberg, M. (1977). DNA content in the genus *Xenopus*. *Chromosoma* **59**:253-257.

Tinsley, R. C. (1973). Studies on the ecology and systematics of a new species of clawed toad, the genus *Xenopus*, from western Uganda. *Journal of Zoology, London* **169**:1-27.

Tinsley, R. C. (1975). The morphology and distribution of *Xenopus vestitus* (Anura:Pipidae) in Central Africa. *Journal of Zoology, London* **175**:473-492.

Tinsley, R. C. (1981a). The evidence from parasite relationships for the evolutionary status of *Xenopus* (Anura: Pipidae). *Monitore Zoologico Italiano***19**:367-385.

Tinsley, R. C. (1981b). Interactions between *Xenopus* species (Anura: Pipidae). *Monitore Zoologico Italiano* **9**:133-150.

Tinsley, R.C. 1996. "Parasites of *Xenopus*." Pp. 233-251 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Tinsley, R.C. and Jackson, J.A. (1998a). Correlation of parasite speciation and specificity with host evolutionary relationships. *International Journal for Parasitology* **28**:1573-1582.

Tinsley, R.C. and Jackson, J.A. (1998b). Speciation of *Protopolystoma* Bychowsky, 1957 (Monogenea: Polystomatidae) in hosts of the genus *Xenopus* (Anura: Pipidae). *Systematic Parasitology* **40**:93-141.

Tinsley, R.C. and Kobel, H.R., eds. 1996. The Biology of *Xenopus* 1st ed. Oxford: Clarendon Press.

Tinsley, R.C., Kobel, H.R. and Fischberg, M. (1979). The biology and systematics of a new species of *Xenopus* (Anura:Pipidae) from the highlands of Central Africa. *Journal of Zoology, London* **188**:69-102.

Tinsley, R.C., Loumont, C. and Kobel, H.R. 1996. "Geographical distribution and ecology". Pp. 35-59 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Tymowska, J. (1977). A comparative study of the karyotypes of eight *Xenopus* species and subspecies possessing 36-chromosome complement. *Cytogenetics and Cell Genetics* **18**:165-181.

Tymowska, J. and Fischberg, M. (1980). The karyotype of *Xenopus wittei* Tinsley, Kobel and Fischberg, another tetraploid anuran species (Pipidae). *Cytogenetics and Cell Genetics* **28**:208-212.

Visser, S. A. (1962). Chemical investigations into a system of lakes, rivers and swamps in Southwestern Kigezi, Uganda. *East African Agricultural Forestry Journal* **28**:81-86.

Vonwyl, E. and Fischberg, M. (1980). Lactate dehydrogenase isozymes in the genus *Xenopus*: Species specific patterns. *Journal of Experimental Zoology* **211**:281-290.

Willig, M.R., Owen, R.D. and Colbert, R.L. (1986). Assessment of morphometric variation in natural populations: the inadequacy of the univariate approach. *Systematic Zoology* **35(2)**:195-203.

CHAPTER 5 : *XENOPUS LAEVIS VICTORIANUS* VS. *XENOPUS LAEVIS BUNYONIENSIS:* GEOGRAPHICAL REPLACEMENT OR SYNONYMIZATION?

INTRODUCTION

The geographical area of interest in this chapter is the same as that which formed the focus for chapter 4, the Central African Highlands. The subject is that of taxonomic confusion between morphologically similar *X. l. victorianus* and *X. l. bunyoniensis*. Confusion appears to have been confounded by local geographical replacement of taxa in these highlands over the course of the last century. Evidence for this change is provided by the museum record and highlights a need for care when comparing old with new distribution records (Tinsley, Kobel and Fischberg, 1979). Early records are based on 17 samples (762 specimens), collected between 1913-1939, and show the exclusive occupation of the lakes and water bodies of Kigezi, southwestern Uganda, by *X. l. bunyoniensis* (Tinsley, 1973). Lakes include Mutanda, Mulehe, Bunyonyi and Chahafi. The taxon is also recorded from nearby swamps and streams in Nyakabande near Mutanda, the Kayonsa forest in southwestern Uganda, Bulera in Rwanda and Mamvu in the Eastern DRC (Tinsley, 1973; 1975; Tinsley *et al.*, 1979). Loveridge also refered to a sample of 81 *X. l. bunyoniensis* specimens collected in southwestern Uganda in 1942 (Loveridge, 1942).

Six hundred specimens were collected from Lakes Mutanda and Mulehe in 1969 and examined by Tinsley. These were found to comprise of *X. vestitus* exclusively (Tinsley, 1973), apparently demonstrating the complete replacement of *X. l. bunyoniensis* by *X. vestitus* in these lakes over a period of less than thirty years. A single aberrant specimen was later identified as *X. l. bunyoniensis* in a sample of *X. vestitus* collected in 1975 (Tinsley, 1981b), since when, none has been found. Fieldwork carried out in 1972 and 1975 and further examination of museum collections revealed the presence of another octoploid species, *X. wittei,* in lakes Mulehe and Bunyonyi. The same period of field and museum work revealed the apparent replacement of *X. l. bunyoniensis* by *X. l. victorianus* near lake Bulera (Tinsley, 1981b). This evidence, taken as a whole, shows *X. l. bunyoniensis* to have been replaced by either *X. wittei*, *X. vestitus* or *X. l. victorianus* in each of the lakes of its former occupation. The origins of each of these three replacement taxa have been determined by examination of museum collections made in these highlands early last century, and are outlined in chapter 4.

It is rare that there should be such a well-documented distribution record covering a period of geographical replacement, as is the case in southwestern Uganda. Unfortunately, a gap in the museum record of nearly thirty years covers the precise period of species turnover. The parasite record from

specimens collected at lake Mutanda however, provides indirect evidence of the timing of the replacement event in this lake (Tinsley, 1981b). The tapeworm parasite, *Cephalochlamys namaquensis* (which infects *X. l. bunyoniensis* but not *X. vestitus*, now prevalent in this lake) was prevalent in a sample of *Xenopus* specimens collected there in 1958 but absent from a sample collected in 1965. Its absence has been explained in terms of the host species having been replaced in this lake. That an isolated *X. l. bunyoniensis* specimen was caught in this lake in 1975, should not be dismissed however.

Taxonomic confusion

Documentation of the history of occupation of lakes by these taxa is complicated by there having been a considerable degree of taxonomic confusion. A number of factors have contributed to this, resulting at times in up to four designations being applied to a single taxonomic form (Tinsley, 1973; 1975). Much overlap occurs between species ranges in an area of highly complex topography (Tinsley, 1981), each having shown recent evidence of considerable redistribution (Tinsley *et al.*, 1979). Furthermore, octoploids were not formally recognized until the 1970's, and had until then been attributed to a different taxon. Although retrospectively, these forms are each morphologically distinguishable from each other and from each of the two *X. laevis* taxa, morphological similarity between *X. laevis* taxa continues to augment confusion.

The original *X. l. bunyoniensis* description (Loveridge, 1932) was based on morphology and distinguished the taxon on account of it being smaller than other *X. laevis* sub-species, with a heavily spotted venter. Parker (1932) later examined a small sample of this taxon, provided comprehensive documentation of its morphology and referred the taxon instead to the nominate form, *X. l. laevis*. The taxon is said to have had large prominent eyes, a short snout and wide head, however Parker dismissed the taxonomic significance of each of the diagnostic characters used by Loveridge, explaining them instead within a biological context (Parker, 1932). The small mean size, he attributed to a probable history of malnutrition, supported by field observations of a pelagic existence, reported by Worthington (see Parker, 1932). The specimens' sexual immaturity lent weight to his argument that the sample may represent an under-developed form. The wide head and bulging eyes could be explained by a heavy burden of encysted metacercariae affecting the lower eyelid of specimens, causing eyes to bulge and giving the illusion of a wider head and shorter snout. Furthermore, this parasite infection is likely to have damaged the eye, causing pressure to build up which would have affected vision, and consequently feeding behaviour. Evidence of compromised vision affecting feeding behaviour is however, disputed by Elepfandt (1996). Blackler and Fischberg (1968) also challenge the taxonomic integrity of *X. l. bunyoniensis*, referring it instead to *X. l. victorianus*, but without giving reasons.

Tinsley later examined a sample collected in 1935 from lake Bunyonyi. In a comparative analysis, which included a 1925 sample of specimens with very low infection levels, he demonstrated that the parasite did indeed distort head shape, and that the heads of both taxa, *X. l. bunyoniensis* and *X. l. victorianus*, strongly resembled one another in the uninfected state (Tinsley, 1973). He also documented morphological variation between *X. l. victorianus* and *X. l. bunyoniensis*, the head of the latter typically being wider at the level of sub-ocular tentacles relative to maximum head width, and having longer limbs and digits than the former (Tinsley, 1973, 1975).

A number of attempts have since been made to determine the taxonomic status of '*X. l. bunyoniensis*' specimens returned live to the laboratory in Geneva, collected from Lakes Bulera and Luhonda, Rwanda during fieldwork in 1975. At the time of collection, Fischberg believed them to be *X. l. bunyoniensis* on the basis of geography and morphology, but the site is now known to be occupied by the morphologically indistinguishable taxon, *X. l. victorianus* (Tinsley, 1981b). Whilst it is almost certain that 1975 specimens from Lake Bulera were actually *X. l. victorianus,* they formed the basis of a series of comparative molecular and biochemical studies comparing them with *X. l. victorianus* collected from Kitanga and Kampala in Uganda and Shama, Kissizi and Mbuye in Rwanda. Despite a high probability that each of these samples was one and the same taxon, results nevertheless warrant assessment. These results are varied. Where distinction of taxa at sub-species level has been possible, comparison of globin expression (Burki and Fischberg, 1986), albumin evolution (Graf and Fischberg, 1986), and lactate dehydrogenase isozymes (Vonwyl and Fischberg, 1980) have each been unable to distinguish between these two *X. laevis* populations, whilst distinction between each of these and any other *X. laevis* sub-species has been possible. Studies comparing haemoglobin (Burki, Schwager and Fischberg, 1984; Muir, 1981) and sperm basic chromosome proteins (Mann, Risley, Eckhardt and Kasinsky, 1982) in contrast, have been able to distinguish between *X. l. victorianus* and *X. l. bunyoniensis*. These studies shall be addressed in the discussion.

AIM

It is the aim of this chapter to present osteological evidence clarifying the taxonomic position of *X. l. bunyoniensis* with respect to that of *X. l. victorianus*, now widespread in the region. The study will incorporate two additional *laevis* sub-species, *X. l. sudanensis* and *X. l. laevis* in order to establish whether osteology is a sensitive enough tool for taxonomic distinction at the sub-species level. Data will be assessed for evidence of osteological distinction between *X. l. bunyoniensis* and *X. l. victorianus*. Characters identified as differing significantly between these taxa will be appraised for their taxonomic significance, drawing on results from analysis of age-related osteological variation

from chapter 3. The ultimate aim of the chapter will be to establish whether osteological evidence supports the view that these taxa should be synonymized.

An account describing the osteology of *X. l. bunyoniensis* will also be presented, and will incorporate results from statistical analysis identifying the primary osteological characteristics of the sample, as compared with those of *X. l. victorianus*.

MATERIALS AND METHODS

All specimens used were determined to be sexually mature. Whilst the arms of many males were darkened, indicating that they were in breeding season and sexually mature, males that lacked these darkened gloves were equivalent in size to those of the same taxon which displayed gloves, and had large testes. Females lacking well-developed ova had convoluted oviducts, suggesting recent evacuation of ova. Further evidence of maturity is provided by osteology (see below).

X. l. bunyoniensis: Origin, Lake Bunyonyi, southwestern Uganda, 1925. All borrowed from the AMNH. The sample comprised 2 males and 3 females.

X. l. victorianus- Origin, various including Chahafi (Uganda), Nyamata (Kigali, Rwanda), Kipoyo (Kivu). All specimens were from the private collection of RCT. Those with prefix A'xx' were from the Salta Museum of Natural History, Argentina. The sample comprised of 7 males and 8 females.

X. l. laevis- An introduced feral population, almost certainly of Cape origin were collected by R. C. Tinsley from Avocado Pond, San Diego, California. The sample comprised 6 males and 14 females.

X. l. sudanensis: Origin Sir, Cameroon. Specimens were collected by J. L. J. Hulselmans, University of Antwerp and comprised of 6 males and 5 females.

Snout-vent length was recorded for each specimen prior to clearing and single-staining, and a total of 60 characters measured, a further 14 scored on the basis of inter-specific variation, following methods outlined in chapter 3. Characters are listed in Appendix 1, chapter 3.

Univariate statistical methods follow those outlined in chapter 3, as do methods testing for measurement repeatability, PCA and DFA. Only measurements that were highly repeatable were used. Methods for PCO follow those outlined in chapter 4.

RESULTS

Listed below are specimens that formed the basis of the following osteological account. Individual identification numbers are included for cross-reference purposes. Sex and snout-vent measures are also provided.

Snout-vent length and sex

X. l. bunyoniensis: A40411 (male; 33.5mm), A40412 (male; 35.7mm), A40421 (female; 40.9mm), A40425 (female; 37.8mm), A40429 (female; 42.6mm).

X. l. victorianus: Ch17(male; 39mm), Ch26(male; 39mm), Ch6 (male; 40mm), Ch10 (male; 45mm), C31 (male; 46mm), A37.3 (male; 47mm), A24.2 (male; 49mm), Ch9 (female; 49mm), Ch38 (female; 49mm), Ch8 (female; 50mm), C37 (female; 54mm), C13 (female; 56mm), A24.4 (female; 62mm), A 37.1 (female; 63mm), A24.6 (female; 44mm).

X. l. laevis: XVI (male, 49mm), 89.12 (male; 51mm), X (male; 55mm), XIII (male; 58mm), t (male; 66mm), gjmII (male, 71mm), V (female, 55mm), q (female; 55mm), g (female; 76mm), j (female; 77mm), 89.3 (female; 77mm), IV (female; 78mm), 89.02 (female; 81mm), 89.11 (female; 81mm), 89.05 (female; 82mm), d (female; 82mm), 89.07 (female; 84mm), B (female; 85mm), h (female; 85mm), 89.01 (female; 96mm).

X. l. sudanensis: S5 (female; 62mm), S7 (female; 65mm), S8 (female; 63mm), S9 (male; 50mm), S10 (male; 49mm), S12 (female; 55mm), S14 (female; 55mm), S17 (male; 50mm), S20 (male; 40mm), S21 (male; 47mm), S22 (male 46mm).

Osteological description of *X. l. bunyoniensis*

Cranium

Frontoparietal

The element is either parallel-sided or slightly waisted, and is considerably wider at its point of lateral association with occipitopetrosals than at anterolateral corners (Illustration 5-1). Its caudal profile is rounded, bears paired posterolateral processes and extends short of the foramen magnum margin. The apex is obtuse. Rostral margins may be continuous or discontinuous. Anterolateral corners are moderately well-pronounced in most specimens, whilst being rounded in others. Parasagittal crests are indistinguishable in some specimens, whilst in others they form a narrow skull table, are parallel and diverge at the level of the pineal foramen. Crests extend towards anterolateral corners of the element and converge to form a narrow U-shape within the posterior frontoparietal margin. Brain-case walls are slightly convex in the region of cerebral hemispheres. The pineal foramen occurs approximately one third of the way along the element from the apex tip.

Sphenethmoid and vomer

The sphenethmoid is parallel-sided or slightly waisted with laterally-displaced notches, representing orbitonasal foramina, occurring along the rostral margin (Illustration 5-2). Retractor bulbi muscle scars (rtbs) are weak, and not always distinguishable. Where discernable, the gap separating scars is small. Optic foramina occur ventrolaterally within rtbs, are large in all specimens, generally measuring up to one fifth the length of the main element body (exception- small in A40429). Posterior margins of scars extend towards posterolateral alae, which are no wider than the main sphenethmoid body. There is no ridge connecting alae, beyond which the element narrows to form a parallel-sided, caudal process that extends just short of the foramen magnum margin.

The moderate-sized vomer is azygous in all specimens examined and is edentulous. The element is rhomboid in shape with bilaterally convex anterior margins and convex posterior margins.

Occipitopetrosal

Occipitopetrosals are stocky (Illustration 5-1, Illustration 5-2). Posterior margins are oriented obliquely and anterior margins perpendicular with respect to the main body axis, converging laterally on cristae parotica. Cristae parotica are varied in relative developmental state throughout the sample, from being absent in some (A40425) to moderately well-developed in others (A40429), but are small in most. Occipitopetrosals remain medially unfused, despite their caudal-most margins being in close association. The dorsal surface is convex except for a reniform epiotic eminence, which occurs in various states of development, from rounded in some to pronounced diagonal ridge in others. Ventrally, Eustachian troughs are deep, the ridges along their posterior margins directed posteromedially, and processes associated with these ridges small.

Pterygoid

Anterior and lateral rami of the pterygoid lack any distinguishing features (Illustration 5-1, Illustration 5-2). The ventromedially directed flange margin is smooth, and distal flange moderately curved. Asymmetrically bifid medial rami are separated by a moderately small inter-otic gap. Paired anteromedially-directed alae are small and tapered, whilst medially directed alae are unevenly tapered, their posterior margins perpendicularly oriented with respect to the main body axis.

Tympanosquamosal and columella

The round annulus lacks an otic process to support the pars medial plectra of the columella at its pivot-point (Illustration 5-1). The slender squamosal arm extends rostrally; its site of divergence from the dorsal face of the annulus is generally V-shaped. A well-developed zygomatic ramus associates closely and moderately robustly with the dorsal face of the anterior pterygoid ramus. The stalk at the medial base of the tympanosquamosal is short in females, absent in males and does not overlap occipitopetrosals at all, indeed a cartilaginous gap separates the two.

Columellae are long, narrow and lack any form of elaboration of the pars media plecra at its pivot point. The element is generally longer posterior than anterior from its pivot point, though in specimens with better-developed cristae parotica, anterior and posterior components of the element are approximately equal in length.

Maxillary arch

The maxillary arch is short, extending only one third the way across the orbit and has a dental row that is almost as long as the arch itself (Illustration 5-2). Tooth numbers average 35 (range: 33-38). Maxillaries are distally tapered. The pars palatina is moderately deep and the facial flange narrow. Alary processes are terminally bifid.

Mandible

As in chapter 2.

Nasal

The rostrally directed nasal component reaches the anterior skull margin, is parallel-sided, un-cleft and lacks a medial scar in all but two of the specimens examined (A40412, A40421) (Illustration 5-1). Ventral curvature is modest and lateral extensions lack foramina. Anterior margins of lateral extensions are perpendicular/sub-perpendicular with respect to the main body axis and are evenly concave in the region where they meet the rostral extension in all but one of the specimens examined, in which small, paired, rostrally-directed prominences interrupt an otherwise smooth profile. Septomaxillaries lack lacrimonasal foramina.

Calcification of rostral cartilaginous elements

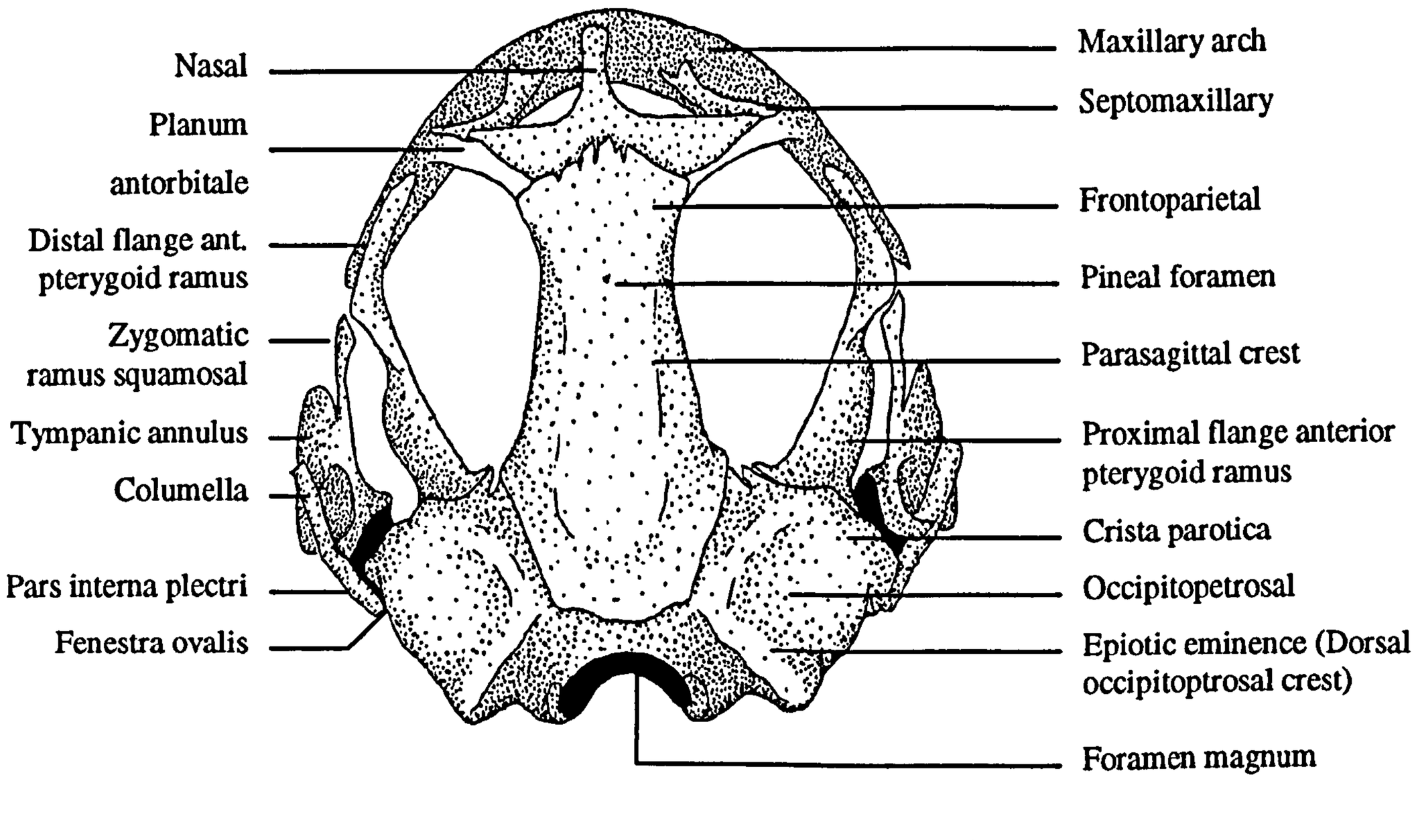
Calcification of rostral elements varies from none at all (A40425), through to quite extensive in others (A40429), indicating a range of post-maturation developmental stages. Calcification affects planum antorbitale, nasal floating cartilage, nasal rostral cartilage, septum nasi and alary cartilage.

Degree of fusion between cranial elements

None.

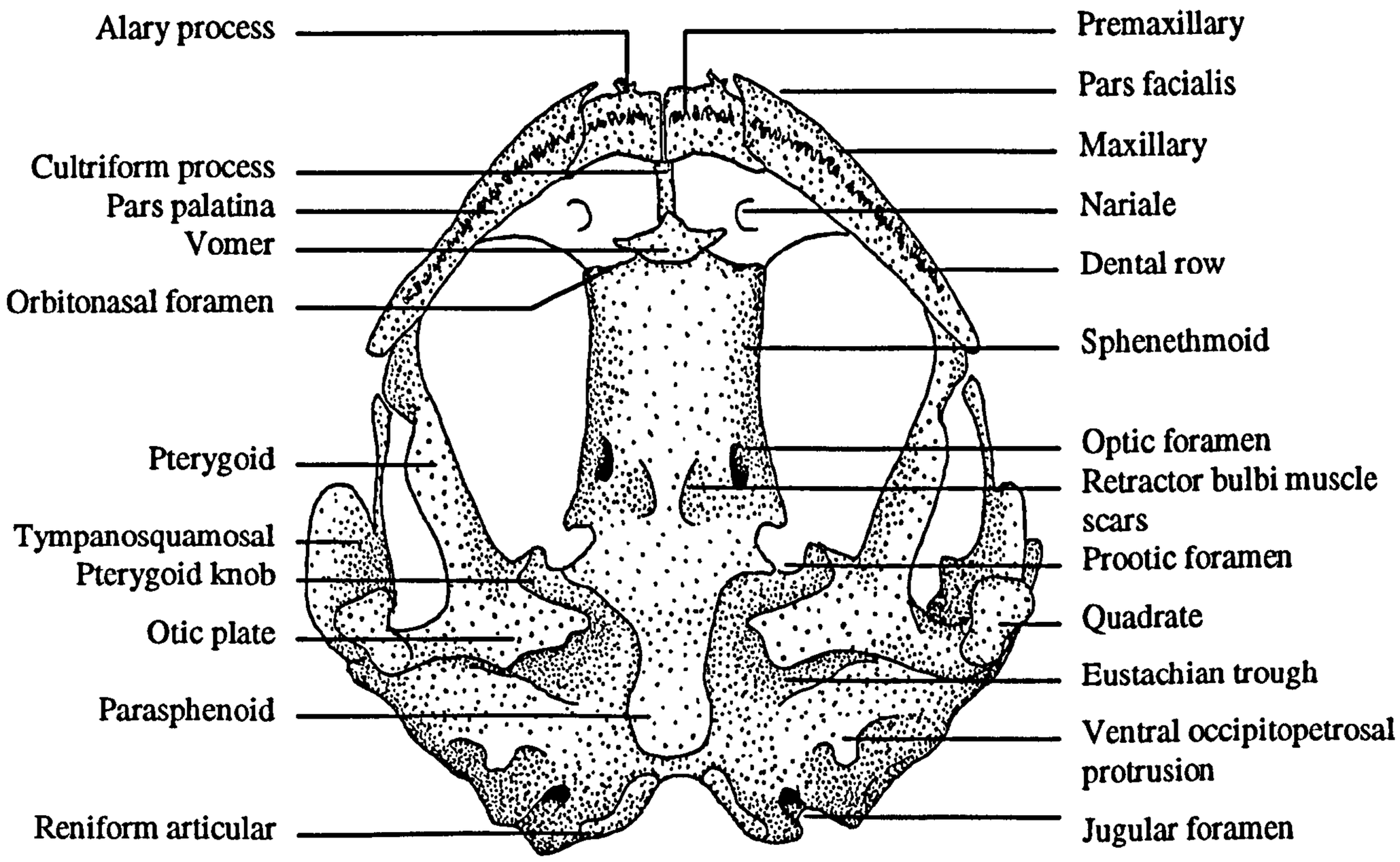
Hyolaryngeal apparatus

As in chapter 2 account.



Dorsal view *X. l. bunyoniensis* skull

Illustration 5-1



Ventral view *X. l. bunyoniensis* skull

Illustration 5-2

Axial skeleton

Individual vertebrae are approximately equal in length and width (Illustration 5-3). The post-sacral portion of the column is approximately equal in length to the pre-sacral portion. The atlas bears widely-divergent cotyles and a slightly concave anterodorsal margin, narrowing slightly towards its posterior margin and bearseither a single, double or no dorsal spines. Other pre-sacral vertebrae are variable in their degree of dorsal ornamentation. Almost all specimens lack dorsal spines on the anterior-most vertebrae; in some, single spines occur on neural arches nearer the sacrum (A40411, A40429). Equally, posterior margins of vertebrae near the sacrum may bear discontinuous posterior margins.

Transverse processes are well-developed, though are remarkably slender. The first pair is moderately short, is widest at mid-length or parallel-sided, and angled perpendicularly to the vertebral column. The second pair is the longest, perpendicular or anterolaterally directed on leaving the column, is evenly curved along their length and posterolaterally directed. The third pair, only marginally shorter than the second, is generally perpendicular on leaving the column, curving posterolaterally. Curvature is strongest proximally and there is a well-developed cartilaginous cap at the terminus of each, extending towards sacral diapophyses.

Ventrally, the atlas bears a convex, medially-notched anterior margin. Articular faces between centra are wide, and spinal foramina between pedicels correspondingly small. Sacral diapophyses are approximately symmetrical around the transverse plane, though some are slightly expanded in a posterior direction. All are parallel-sided.

Anterior appendicular skeleton

Pectoral Girdle

Cleithrum and suprascapula are large, overlapping mid-dorsally and associating laterally with a short scapula. Partes acromialis and glenoidialis of the scapula are found in both cleft and un-cleft conditions within the sample. In the former case, there may or may not be a scar marking this site of fusion. Clavicles arch anteromedially, their medial margins measuring up to three times the lateral width of the element. The angle formed between anterior margins of these elements is acute, and posterior margins highly obtuse, almost straight. The cleithrum is as wide medially as it is laterally, the gap separating them medially measuring between one and two times the width of their medial margins. The cleithrum forms a low mean angle of 44° to the main zonal axis.

Prevalence of calcification along the zonal plane and on suprascapula cartilage is very low, occurring to a small degree in only one of the specimens examined (A40429).

Forelimb

Although conforming in gross morphology to the account given in chapter 2, the arm and carpal elements of many specimens in this sample are characterized by under-ossification of condyles, carpal elements and phalanges.

Posterior Appendicular Skeleton

Pelvic Girdle

The pelvic girdle conforms that described in chapter 2 but all elements consistently remain unfused in the region of the inter-iliac symphysis with ilia extending in parallel from this region.

Hindlimb

Again, elements conform to the gross morphology outlined in the account in chapter 2, but are commonly characterized by under-ossification of cotyles, tarsal elements and phalanges. Whilst the relative proportion of proximal-to-distal prehallux elements is approximately 1:1, these elements are relatively small and both are roughly oval in shape.

Sexual dimorphism

Males differ from females in having a more dorsally-oriented tympanic annulus, in lacking a medial tympanic stalk, in having slightly larger tympanic annuli and correspondingly, a short squamosal arm. Muscle scars are also slightly less pronounced in males. The male hyolaryngeal apparatus is well calcified whilst that of females is normal.

Age-related Variation

By reference to chapter 3, a number of characters appear to be underdeveloped whilst others are indicative of maturity. These are listed below.

Cranium

Immaturity: large optic foramina which open ventrally, prominent epiotic eminence and short crista parotica.

Maturity: robust zygomatic process of squamosal, absence of nasal scars, position of vomer over anterior sphenethmoid margin and calcification of the male larynx.

Vertebral column

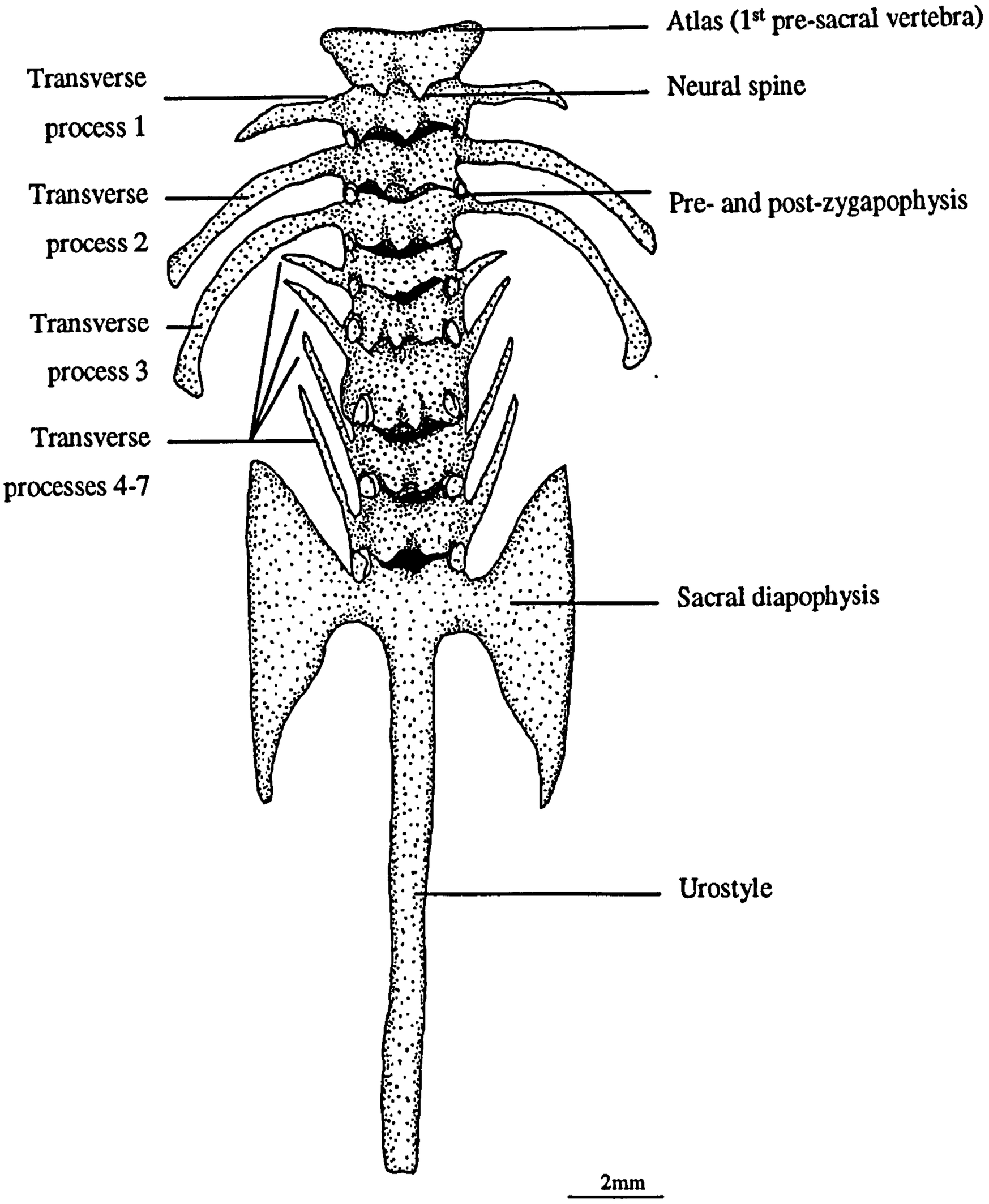
Immaturity: no spinous processes and symmetrical sacrum.

Maturity: divergent cotyles on atlas and concave anterodorsal margin, well-developed transverse processes, closed spinal foramina and ratio of pre- to post-sacrum length lass than unity.

Anterior appendicular skeleton

Immaturity: under calcification of phalangeal and proximal carpal elements.

maturity: calcification along pectoral girdle zonal-plane.



Dorsal vertebral column *X. l. bunyoniensis*

Illustration 5-3

Statistical analysis of osteometric data

Results from one-way analysis of variance showed mean snout-vent length of *Xenopus l. laevis* to be significantly greater than that of the other three sub-species (F=23.65, d.f.=3,47, p=0.000), and although mean snout-vent length of *X. l. bunyoniensis* was less than for all other taxa in the analysis, a post-hoc Tukey test showed it to vary significantly only from *X. l. laevis* (F=29.5, d.f.=1,23, p=0.000) (Table 5-1). Males were significantly smaller than females in all cases except for *X. l. bunyoniensis* (see Table 5-2), where the snout-vent length difference between sexes was not significant, possibly confounded by small sample size.

Species	Sex	N	Mean±s.d.
X. l. victorianus	Pooled	15	49±7.4
	Male	7	44±4.16
	Female	8	53±6.7
X. l. bunyoniensis	Pooled	5	38±3.8
	Male	2	34±1.7
	Female	3	40±2.4
X. l. sudanensis	Pooled	11	53±7.9
	Male	6	47±3.6
	Female	5	60±4.5
X. l. laevis	Pooled	20	72±13.7
	Male	6	58±8.6
	Female	14	78±10.9

Table 5-1

Snout-vent length (mm) for each species

	T	d.f.	P
X. l. victorianus	-3.46	11	0.005
X. l. bunyoniensis	-3.2	2	0.085
X. l. sudanensis	-5.25	7	0.002
X. l. laevis	-4.33	12	0.001

Table 5-2

Significance levels of difference in snout-vent length between sexes for each species

Multivariate statistics were applied to identify the form and relative significance of inter-taxon osteological variation, using data for all measured or scored osteological variables. Sexes were pooled for analysis owing to small sample size but were identified by different symbols in the final Principal Coordinate Analysis scatter graph. This enabled assessment of the magnitude of sexual dimorphism within the context of inter-specific variation. An account of sexual dimorphism was however incorporated into the account describing osteology of *X. l. bunyoniensis* (see above).

Given the high ratio of variables to repeats (comparable to those of Lessa and Wake, 1992), it was necessary to reduce the number of continuous variables in the analysis whilst retaining most of the original information. In order to achieve this, categorical and continuous variables were treated separately. Categorical data were reduced by removing characters identified by Logistic regression as contributing least to inter-taxon variation. Continuous variables were filtered using both Principal Component (PCA) and Discriminant Function Analysis (DFA, using taxon as grouping variable). Characters with lowest component weightings and highest Wilk's λ values respectively were selected for removal. PCA was subsequently used to reduce data to a small number of factors, each representing a linear combination of variables, differentially weighted so as to account for most of the variance in the original data set (Dillon and Goldstein, 1984). The first factor accounted mainly, but not exclusively, for size variation, with all coefficients being of similar, high magnitude and positive sign, except for the value corresponding to the angle separating clavicle and coracoid, which becomes relatively smaller with overall growth. Remaining factors accounted for shape variation. PCA1 explained 85% of the total variation identified by the analysis (Table 5-3).

PCA factor	Cumulative % variation
1	85.2
2	88.1
3	90.3
4	92.1
5	93.2

Table 5-3

Cumulative percentage variation explained by PCA1-5.

Principal coordinate analysis (PCO) was carried out on PCA factors 1-5 and the pair of categorical variables selected by logistic regression as being important taxonomic discriminators (presence/absence orbitonasal foramina and medial stalk at base of tympanic annulus). Results are presented as a scatter graph in Figure 5-1.

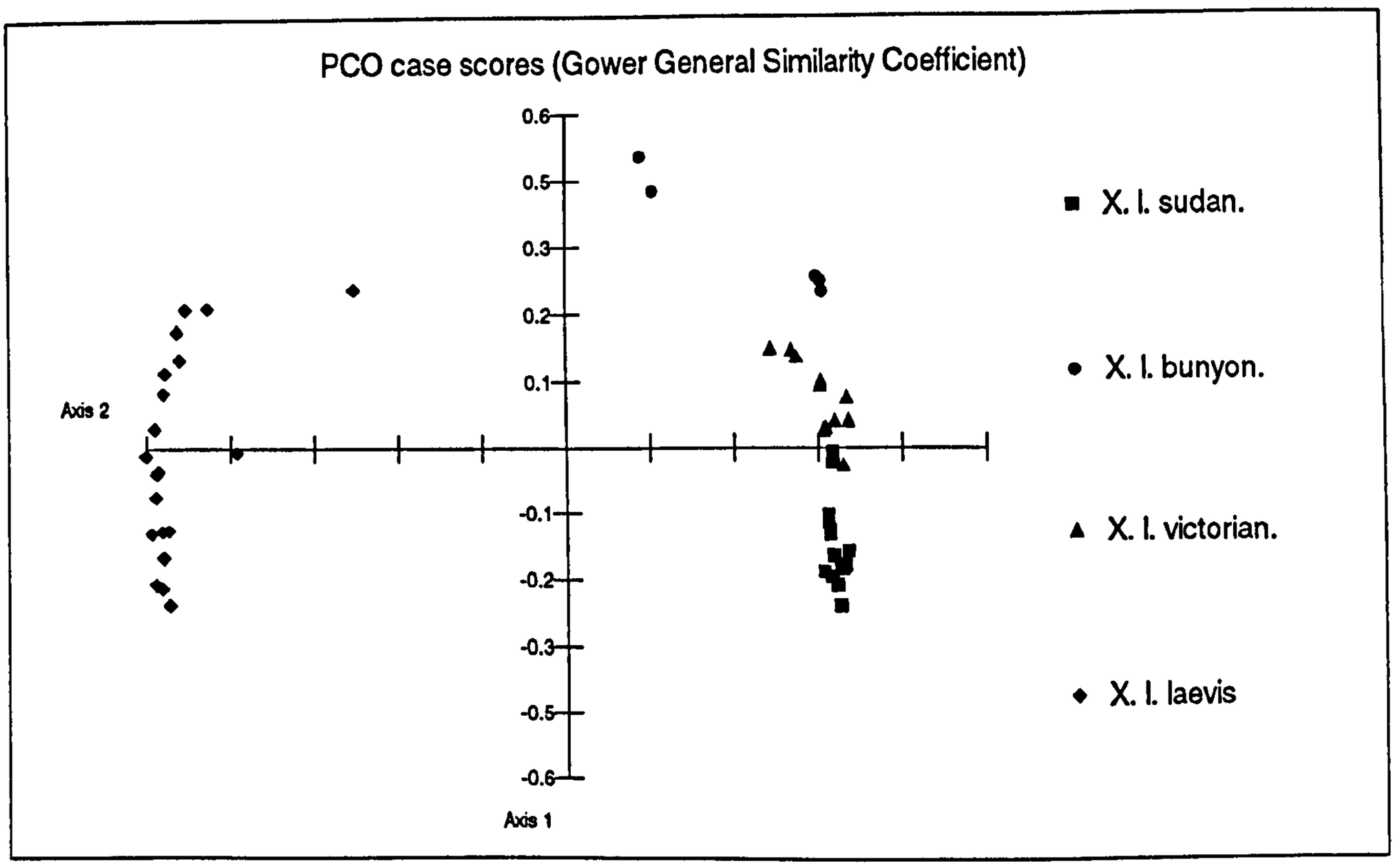


Figure 5-1

Scatter plot of PCO results (analysis of PCA1-5 and character numbers 38 and 45). Sexes pooled.

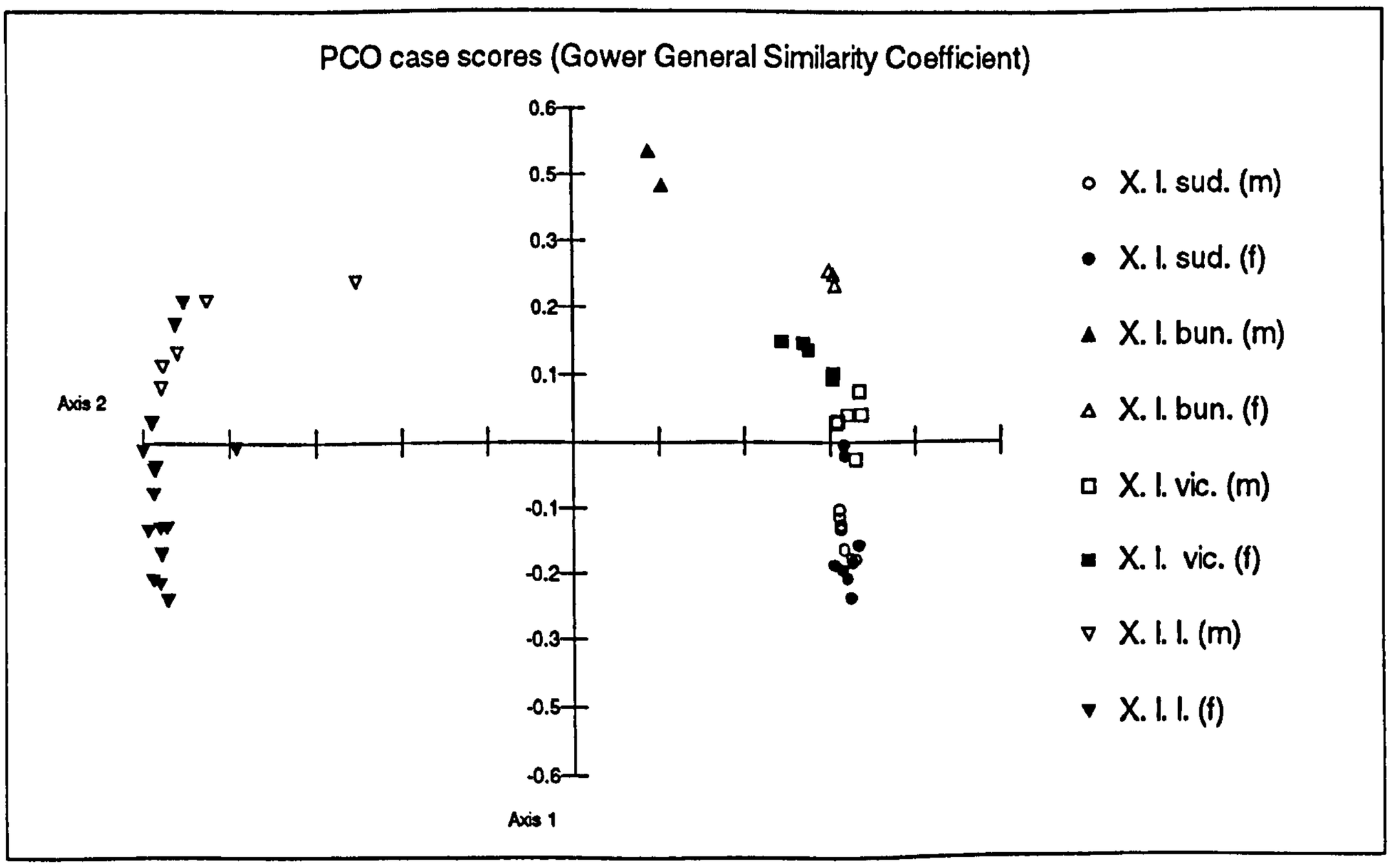


Figure 5-2

Scatter plot of PCO results (analysis of PCA1-5 and character numbers 38 and 45). Sexes plotted separately.

To assess the significance of size within the context of overall osteological variation, PCO was also carried out on PCA2-5 and data for the pair of categorical variables, excluding PCA1, the size factor. Results from size-out PCO analysis differed little from those represented in Figure 5-1 and showed that *Xenopus l. bunyoniensis* is phenetically distinct from *X. l. laevis*, *X. l. victorianus* and *X. l. sudanensis* on account of factors other than size alone.

In order to identify the main characters accounting for inter-taxon variation (excluding those aspects encompassed by categorical variables, which are described in the osteological description), DFA was carried out on PCA factors 1-5, with taxon as the grouping variable. This analysis was conducted on the two taxa of biogeographical interest, *X. l. bunyoniensis* and *X. l. victorianus* alone, since it was intended purely as a means of identifying characters that can be used to distinguish between these two taxa, in order to assess their evolutionary significance. The Rao's F value corresponding to each factor was taken as an indicator of that factor's discriminatory importance. Rao's F was chosen in preference to Wilk's λ owing to small sample size (see Barlow, Jones and Barratt, 1997) (Table 5-4).

PCA factor number	1	2	3	4	5
Rao's F	7.563264	27.49003	0.234248	0.006559	0.002501

Table 5-4

Rao' V values corresponding to each of factors 1-5

Factor 2 explains most of the inter-taxon variation. Cross-reference to character loadings in PCA for factor 2 listed the characters in Table 5-5 as being most discriminatory between *X. l. victorianus* and *X. l. bunyoniensis*. T-tests were carried out on each of these characters, standardized against snout-vent length (except in the case of tooth counts), to describe the form and degree of variation existing between *X. l. victorianus* and *X. l. bunyoniensis*, relative to snout-vent length.

	t	d.f.	*p<0.05, **p<0.01, ***p<0.001
Number of maxillary teeth	6.68	6	***
Prepollex length	-0.13	9	
Skull length	11.4	12	***
Length between medial margins of clavicle and coracoid	-3	14	**
Length maxillary dental ridge	8.55	8	***
Depth maxillary arch	9.09	17	***
Number of premaxillary teeth	3.04	5	*
Length distal prehallux element	-3.03	13	**
Distance between frontoparietal and foramen magnum	6.98	15	***
Length proximal prehallux	-2.17	7	
Length columella	7.75	15	***
Width foramen magnum	4.72	5	*
Depth maxillary arch from level of premaxillary dental row	6.77	7	*

Table 5-5

-/+ sign preceding t-values refers to character size in *X. l. bunyoniensis* relative to that of *X. l. victorianus*. The magnitude of inter-taxon variation for each character is reflected by differences in relative t values.

In summary, *X. l. bunyoniensis* differs from *X. l. victorianus* mainly on account of differences in upper jaw morphology. *Xenopus l. bunyoniensis* bears more maxillary and premaxillary teeth, a longer maxillary, deeper maxillary arch and longer dental row than that of *X. l. victorianus*. In addition to these characters, the skull and columella of *X. l. bunyoniensis* are considerably longer than those of *X. l. victorianus*, the foramen magnum wider with a wider gap separating this element from the frontoparietal. The pectoral girdle is small. Prehallical elements, though similar in relative proportion in each taxon, are much smaller in *X. l. bunyoniensis*, as is the prepollex, relative to that of *X. l. victorianus*.

DISCUSSION

Morphology

Whilst the morphological variation documented by Loveridge (1942) as separating *X. l. victorianus* and *X. l. bunyoniensis* has been explained within a biological context (Parker, 1932), no alternative interpretation has yet been presented regarding morphological variation documented by Tinsley (1973). It is difficult to assess the significance of inter-taxon variation in relative bone lengths identified by Tinsley (1973), since no error values were included with his results. The taxonomic value of differences in relative limb length, when measured externally, is questionable in a sample of animals known to have been undernourished, given that specimens would have been skeletonized, exposing a greater proportion of the limb than had they been of a more robust, stout build. Similarly, greatest head width is influenced by the amount of fat stored posterior to tympanic annuli. If *X. l. bunyoniensis* were undernourished, skull width at the level of tympanic annulus would have been reduced relative to width at the level of eyes, possibly explaining differences in these relative measures between these two taxa. In this respect, earlier documentation of *X. l. bunyoniensis* having a wide head (Parker, 1932) are in agreement with subsequent observations documented by Tinsley (1973), who regarded head width at the level of eyes to be wide compared with that of *X. l. victorianus*.

Visual impairment

Evidence from studies by Elepfandt (1996) disputes the possibility that visual impairment influences feeding efficiency. However, it is known that blind or visually impaired specimens are generally characterized by 'liberally dark-spotted lower surfaces' (Hogben and Slome, 1931: see Parker 1932), one of the characteristics documented for *X. l. bunyoniensis*. Whilst it is possible that this is a genetically determined trait, it remains plausible that it is the outcome of impaired vision. That all published accounts documenting the morphology of this taxon indicate malnutrition is consistent with reduced food intake in these specimens, however impaired vision need not necessarily have been a causal factor.

Molecular and biochemical studies

Despite a high degree of overall similarity, small differences in elecrophoretic patterns between sperm basic proteins of *X. l. bunyoniensis* and *X. l. victorianus* are probably the result of different preparation techniques. Proteins from the **testes** of *X. l. bunyoniensis* were compared with proteins from the **sperm** of *X. l. victorianus* and shared identical patterns of band migration, with the addition, in the case of *X. l. bunyoniensis*, of an extra band. The **testes** of *X. l. sudanensis* were also used instead of **sperm**, and an extra band, matching that seen in *X. l. bunyoniensis*, was also present,

providing support for my theory that proteins originating in the testes accounted for the additional band that is said to distinguish between *X. l. bunyoniensis* and *X. l. victorianus*. If indeed this were the case, it is my opinion that the **sperm** basic chromosomal proteins of *X. l. bunyoniensis* would be otherwise indistinguishable from those of *X. l. victorianus*.

Results from haemoglobin studies have also provided evidence of the distinct taxonomic status of *X. l. victorianus* and *X. l. bunyoniensis*. It may however be pertinent to repeat that both samples used in this comparison were collected in 1975 at different sites within the geographical range of *X. l. victorianus*. It is possible that each sample represented a different geographical variant of *X. l. victorianus*, especially since prior to collection of this sample no *X. l. bunyoniensis* had been collected in this region for over 25 years. Not only could geographical variation have accounted for these differences, but also, and perhaps more likely, altitude differences in their respective habitats could have altered the physiological properties of haemoglobin, as has been documented for a number of other groups; coots (Leon *et al.*, 1993), pumas and foxes (Leon-Velarde, deMuizon, Palacios, Clark and Monge, 1996), mammals and birds (Leon, 1997), red deer (Pellegrini, Giardina, Castagnola, Olianas, Sanna, Fais, Messana and Corda, 1999) and geese (Lui, Li, Jing, Liang, Kua, and Lu, 2001). No data were available for the effects of altitude on anuran haemoglobin. Further research is needed in order to clarify the position of *X. l. victorianus* in this respect.

Osteology

An osteological description of *X. l. bunyoniensis* is presented for the first time, and incorporates results from statistical analysis of osteometric data. These results showed that the methods employed were generally adequate for the separation of taxa at the sub-species level, although osteological variation showed there to be no significant difference between *X. l. sudanensis* and *X. l. victorianus*, separated in the original account on the basis of inter-narial distance and number of lateral line plaques (Perret, 1966), and generally considered to be very closely related (Kobel *et al.*, 1998). *Xenopus l. bunyoniensis* was clearly separated from the *X. l. victorianus* and *X. l. sudanensis* cluster and also from *X. l. laevis*, providing preliminary evidence of its distinct phenetic status, specifically with respect to *X. l. victorianus*. The degree of variation separating some *X. l. bunyoniensis* specimens from *X. l. victorianus* is approximately the same as that which separates *X. l. victorianus* from some *X. l. laevis* specimens, for which there is good evidence of taxonomic distinction (Graf, 1996; Kobel *et al.*, 1998). Whilst sexual dimorphism is described for *X. l. bunyoniensis* (see osteological description), PCO analysis of inter-species osteological variation generally showed sexes to cluster together, indicating that the magnitude of sexual dimorphism was not significant within the context of inter-taxon variation.

The taxonomic significance of characters identified by DFA as being of primary importance in the distinction between *X. l. bunyoniensis* and *X. l. victorianus* will be addressed in turn, in the

light of available information from the literature. The additional possibility that *X. l. bunyoniensis* represents a sub-adult population of *X. l. victorianus*, as is implied in the literature (see Parker, 1932), will also be considered, drawing on results from chapter 3.

Tooth numbers are known to increase throughout life in amphibians (Lessa and Wake, 1992), yet the ranges observed for this character in these two taxa are non-overlapping, mean tooth-number in *X. l. bunyoniensis* exceeding that of *X. l. victorianus* by nine. Accepting the risk in drawing cross-taxon comparisons, maximum variation in tooth number between youngest and oldest, smallest and largest *X. l. laevis* specimens (chapter 3) was only four teeth, implying that observed variation between *X. l. bunyoniensis* and *X. l. victorianus* exceeds that which could be attributable solely to inter-taxon age-related variation. Furthermore, the direction of variation indicates that, if these two samples actually represent the same taxon, it is unlikely, on the basis of tooth counts, that '*X. l. bunyoniensis*' represents a sub-adult form of *X. l. victorianus*. Evidence of osteology also contradicts suggestions in the literature that the 1925 sample of *X. l. bunyoniensis* was young and sexually immature (Parker, 1932) (see also reference to characters that differentially suggest maturity or immaturity, presented in the section following the osteological description). Whilst evidence suggests that the inter-taxon variation observed for tooth counts between these two taxa is taxonomically significant, it is possible that this is the result of samples having been collected at an interval of fifty years. The morphology of the genus *Xenopus* is renowned for its conservatism however (Kobel, Loumont and Tinsley, 1996), and is unlikely to have changed to this degree over such a short period of time.

Although no reference could be found in the literature to development of the maxillary arch in pipids past 6 months post-metamorphosis, my own results demonstrated a significant decrease in relative depth of the maxillary arch with age in *X. l. laevis*. If *X. l. victorianus* and *X. l. bunyoniensis* are indeed one and the same taxon, the longer maxillary arch seen in the latter relative to that of the former, again supports the view that *X. l. bunyoniensis* represents an older sub-set of the population.

The skull of *X. l. bunyoniensis* is long relative to that of *X. l. victorianus*. Whilst again, data are lacking for changes in this character past 6 months post-metamorphosis, my own results for *X. l. laevis* indicated continued change up until sexual maturity, skulls becoming progressively wider with age. Results from the present study for this character indicate that *X. l. bunyoniensis* represents a young form of *X. l. victorianus*, contradicting indications from data for tooth counts and maxillary arch length. No reference could be found in the literature relating to ontogenetic variation in pipids for other characters that have been shown to differ (non-significantly) between *X. l. victorianus* and *X. l. bunyoniensis*. My own results (chapter 3) however, show none of these to vary significantly with age.

An alternative explanation for observed inter-taxon variation between *X. l. victorianus* and *X. l. bunyoniensis* is that the latter sample was malnourished, as has been suggested in the literature

(Parker, 1932). The affect of starvation on the osteology of amphibians is unknown, and whilst this possibility should not be dismissed, neither can its affects be assessed.

CONCLUSION

If arguments are accepted that explain morphological variation between *X. l. victorianus* and *X. l. bunyoniensis* within a biological context, and results from molecular and biochemical studies in terms of methodological inconsistencies (sperm basic proteins) and natural physiological variation (haemoglobin), published cases in favour of distinction between these two taxa should be dismissed. The possibility that existing samples represent underdeveloped forms of *X. l. victorianus* is also disputed, on account of osteological indications to the contrary. It should be borne in mind however, that specimens used in molecular and biochemical studies may indeed have represented geographical variants of the same taxon, in which case no significant differences would be expected.

Despite these arguments favouring synonymization, results from the present study of comparative osteology have indicated a degree of taxonomic separation that is comparable to that which separates *X. l. laevis* from *X. l. victorianus*, a taxonomic separation that is well corroborated in the literature. The main characters that distinguish *X. l. bunyoniensis* from *X. l. victorianus* are not considered to be ontogenetically variable, and the degree of variation observed for these characters is greater than that which would be expected of intra-specific variability. Osteological evidence is interpreted as indicating that the 1925 sample of *X. l. bunyoniensis* represents a distinct *X. laevis* sub-species which, according to published accounts of changes in distribution, has since been replaced in the lakes of southwestern Uganda and is unknown anywhere else. Only further research into the affects of starvation on the osteology of amphibians will provide the information necessary to discount reservations regarding this interpretation of the taxonomic significance of observed osteological variation.

BIBLIOGRAPHY

Barlow, K.E., Jones, G. and Barratt, E.M. (1997). Can skull morphology be used to predict ecological relationships between bat species? A test using the two cryptic species of Pipistrelle. *Proceeding of the Royal Society, London B* **264**:1695-1700.

Bustos, R.P., Caprini, M.F., Kraglievitch, J.L. and Del Corro, G. (1960). Noticia preliminar acerca del yacimiento de anuros extinguidos de puente morales. *Seperata de la revista de la facultad de ciencias naturales de Salta, Argentina.* **Tomo 1, 2**:5-25.

Burki, E. and Fischberg, M. (1985). Evolution of globin expression in the genus *Xenopus* (Anura: Pipidae). *Molecular Biology and Evolution* **2**:270-277.

Burki, E., Schwager, J. and Fischberg, M. (1984). Electrophoretic patterns of haemoglobin in different *Xenopus* species, subspecies and interspecies hybrids. *Experimentia* **40**:59-64.

Blackler, A.W. and Fischberg, M. (1968). Hybridization of *Xenopus laevis petersi* (*poweri*) and *X. l. laevis*. *Revue Suisse de Zoologie* **75(52)**: 1023-1032.

Dillon, W.R. and Goldstein, M. 1984. Multivariate analysis methods and applications. New York Wiley.

Elepfandt, A. 1996. "Underwater acoustics and hearing in the clawed frog *Xenopus*". Pp. 177-191 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Graf, J.-D. and Fischberg, M. (1986). Albumin evolution in polyploid species of the genus *Xenopus*. *Biochemical Genetics* **24**: 821-837.

Graf, J.-D. 1996. "Molecular approaches to the phylogeny of *Xenopus*". Pp.379-388 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Kobel, H.R., Loumont, C. and Tinsley, R.C. (1996). "The extant species." Pp. 9-33 in The biology of *Xenopus*, edited by Tinsley, R.C., and Kobel, H.R. Oxford: Clarendon Press.

Kobel, H.R., Barandun, B. and Thiébaud, ChH. (1998). Mitochondrial rDNA phylogeny in *Xenopus*. *Herpetological Journal* **8 (1)**:13-18.

Leon-Velarde, F., Sanchez, J., Bigard, A.X. (1993). High altitude tissue adaptation in Andean coots: capillarity, fiber area, fiber type, and enzymatic activities of skeletal-muscle. *Journal of Comparative Physiology B* **163(1)**:52-58.

Leon-Velarde, F., deMuizon, C., Palacios, J.A., Clark, D. and Monge, C. (1996). Haemoglobin affinity and structure in high-altitude and sea-level carnivores from Peru. *Journal of comparative biochemistry and physiology B* **113**:407-411.

Leon-Velarde, F. (1997). Changes in whole blood oxygen affinity and egg-shell permeability in high altitude chickens translocated to sea-level. *Journal of comparative biochemistry and physiology B* **118(1)**:53-57.

Lessa, E.P. and Wake, M.H. (1992). Morphometric analysis of the skull of *Dermophis mexicanus* (Amphibia: Gymnophiona). *Zoological Journal of the Linnean Society* **106**:1-15.

Loveridge, A. (1932). New races of skink (*Siaphos*) and frog (*Xenopus*) from the Uganda Protectorate. *Proceedings of the Biological Society of Washington* **45**:113-115.

Loveridge, A. (1933). Reports on the scientific results of an expedition to the southwestern highlands of Tanganyika territory. *Bulletin of the Museum of Comparative Zoology, Harvard.* **74:** 7-35.

Loveridge, A. (1942). Scientific results of the 4th expedition to forested areas in East and Central Africa. *Bulletin of the Museum of Comparative Zoology, Harvard* **91** (**5**):383-384.

Lui, X.Z., Li, S.L., Jing, H., Liang, Y.H., Kua, Z.Q. and Lu, G.Y. (2001). Avian haemoglobin and structural basis of high affinity for oxygen: structure of bar-headed goose aquomet haemoglobin. *Acta Crystallographica Section D-Biological Crystalography* **57**:775-783.

Mann, W., Risley, M.S., Eckhardt, R.A. and Kasinsky, H.E. (1982) Characterization of sperm/spermatid basic chromosomal proteins in the genus *Xenopus* (Anura: Pipidae). *Journal of Experimental Zoology* **200**:173-186.

Muir, A.V. (1981). Comparison of hemoglobins from the genus *Xenopus* (Amphibia Salientia). *Journal of Experimental Zoology* **218**:327-333.

Parker, H.W. (1932). Scientific results of the Cambridge expedition to the East African lakes, 1930-1.-5. Reptiles and amphibians. *Journal of the Linnean Society, London* **38**:213-229.

Pellegrini, M., Giardina, B., Castagnola, M., Olianas, A., Sanna, M.T., Fais, A., Messana, I. and Corda, M. (1999). Low-temperature sensitivity and enhanced Bohr effect in Red Deer (*Cervus elaphus*) haemoglobin: a molecular adaptive strategy to life at high altitude and low temperature. *Journal of comparative biochemistry and physiology B* **260**:667-671.

Perret, J.,- L. (1966). Les amphibians du Cameroun. *Zoologische Jahrbucher Abteilung Fur Systematik Okologie unt Geographie der Tiere* **93**:289-464.

Tinsley, R. C. (1973). Studies on the ecology and systematics of a new species of clawed toad, the genus *Xenopus*, from western Uganda. *Journal of Zoology, London* **169**:1-27.

Tinsley, R. C. (1975). The morphology and distribution of *Xenopus vestitus* (Anura:Pipidae) in Central Africa. *Journal of Zoology, London* **175**:473-492.

Tinsley, R. C. (1981a). The evidence from parasite relationships for the evolutionary status of *Xenopus* (Anura: Pipidae). *Monitore Zoologico Italiano* **19**:367-385.

Tinsley, R. C. (1981b). Interactions between *Xenopus* species (Anura: Pipidae). *Monitore Zoologico Italiano* **9**:133-150.

Tinsley, R.C., Kobel, H.R. and Fischberg, M. (1979). The biology and systematics of a new species of *Xenopus* (Anura:Pipidae) from the highlands of Central Africa. *Journal of Zoology, London* **188**:69-102.

Vonwyl, E., and Fischberg, M. (1980). Lactate dehydrogenase isozymes in the genus *Xenopus*: Species specific patterns. *Journal of Experimental Zoology* **211**:281-290.

CHAPTER 6 : OSTEOLOGICAL DIFFERENTIATION BETWEEN EASTERN AND WESTERN *XENOPUS MUELLERI* FORMS

INTRODUCTION

Xenopus muelleri (Peters, 1844), collected originally from Tete, Mozambique was among the first species of the genus to be described. Its preponderance in lowland savanna accorded for years with the notion that *Xenopus* taxa were easily separable according to differing preferences for habitat type (see thesis introduction; Tinsley, Loumont and Kobel, 1996). For over 150 years the view prevailed that *X. muelleri* comprised a single taxon whose distribution range exceeded that of all other known *Xenopus* species (Tinsley *et al.*, 1996) extending from Burkina Faso in west Africa, in a broad arc to southern Sudan and southwards through the savannas of eastern Africa as far as Lake Lucia, at 28° south (see chapter 7; Poynton, 1962, 1964; Loumont, 1984; Tinsley, 1981b; Kobel, Loumont and Tinsley, 1996; Tinsley, 1996; Tinsley *et al.*, 1996). Although the southern part of the *X. muelleri* distribution range is well documented, little information exists regarding its northeastern extension into Tanzania and replacement by *X. borealis* in northern Kenya (Tinsley, 1981b).

Xenopus muelleri is believed to have the highest temperature tolerance of all *Xenopus* taxa (Poynton, 1964; Tinsley *et al.*, 1996). The taxon is generally restricted to lowland savanna outside zones of rainforest and dry woodland (Tinsley *et al.*, 1996). This habitat preference appears not to be so strong as that reported to constrain forest species to forest however (see chapter 4; Tinsley *et al.*, 1996). A number of accounts have documented cases in which *X. muelleri* has been found outside savanna, occurring sympatrically in rainforest swamps with *X. andrei*, *X. pygmaeus* and *X. epitropicalis*, all forest species (Loumont, 1986), and in every type of aquatic habitat in the Garamba National Park, Democratic Republic of the Congo (D. R. Congo hereafter), although relative numbers confirm a preference for an open savanna habitat (Inger, 1968). Schiotz (1963) even found *X. muelleri* in an isolated forest clearing 72km from savanna. Aberrant records are to be expected however, given the ecological instability of areas modified by agriculture and deforestation, where localized forest clearing creates a mosaic landscape (Tinsley *et al.*, 1996).

Systematics

A range of comparative studies employing molecular, biochemical, DNA and RNA approaches have long established that a robust taxonomic distinction separates the *X. muelleri* group (*X. muelleri*, *X. borealis*) from other *Xenopus* species (Mann, Risley, Eckhardt and Kasinsky, 1982; Burki and Fischberg, 1985; Graf and Fischberg, 1986). Further evidence of a taxonomic division within *X. muelleri sensu stricto* has since been presented and is founded on studies that have

compared mate-call (Kobel *et al.*, 1996) parasite fauna (Kobel *et al.*, 1996; Tinsley, 1996; Tinsley and Jackson, 1998) and rDNA (Kobel, Barandun and Thiebaud, 1998), the latter study suggesting a degree of divergence between eastern and western forms that warrants taxonomic separation at the species level (Kobel *et al.*, 1998). It is not clear however, whether observed differences between eastern and western forms represent extremes along a polytypic cline, or whether they represent two distinct *X. muelleri*-forms whose ranges converge somewhere along the aforementioned distribution arc.

AIMS

The initial aim of the current study is to assess whether variation exists between the osteology of *X. muelleri* samples collected from eastern and western extremes of the taxon's range, and to establish the form and degree of this variation. Additional samples collected from a number of intervening localities across this range will subsequently be incorporated into the study, the ultimate aim being to determine whether osteological variation in *X. muelleri* occurs as a polytypic cline, or whether *X. muelleri* falls into two discrete sibling-taxa whose corresponding distribution margins are located at a point somewhere along the range currently defined for *X. muelleri*. In the event of evidence suggesting the latter, data will be assessed in order to indicate the approximate geographical position of this association. An additional aim of the study is to provide an account describing the osteology of *X. muelleri* and to provide a list of osteological characters that best distinguish the taxon from others in the genus.

MATERIALS AND METHODS

Specimens were collected from Ndumo and Skukuza (Kruger National Park) in southeastern Africa during fieldwork carried out by R. C. Tinsley in 1995 and by R. C. T., Joe Jackson, Louis DuPreez and myself in 1999. Samples used in the current study comprised 30 specimens in total, 18 from Ndumo and 12 from Skukuza. West African material was borrowed from the Musée Royal de l'Afrique Centrale, Tervuren, Belgium. A pooled sample of 21 comprised 12 specimens from Sir (Cameroon), 3 from Gueme (Cameroon) and 6 from Namoundjoga (Togo). West African samples were pooled owing to individual samples being insufficient for the purpose of comparative analysis, but were distinguished apart in graphs and dendrograms in the final analysis. Other samples included 17 specimens from Uvira, Tanganyika, loaned by the Salta Museum of Natural History, Argentina. A sample of 16 specimens from an area close to the Garamba National Park, northeastern DRC was loaned by the Tervuren museum (see above).

Map of Africa showing distribution of *X. muelleri* sites from which material was obtained for the current study

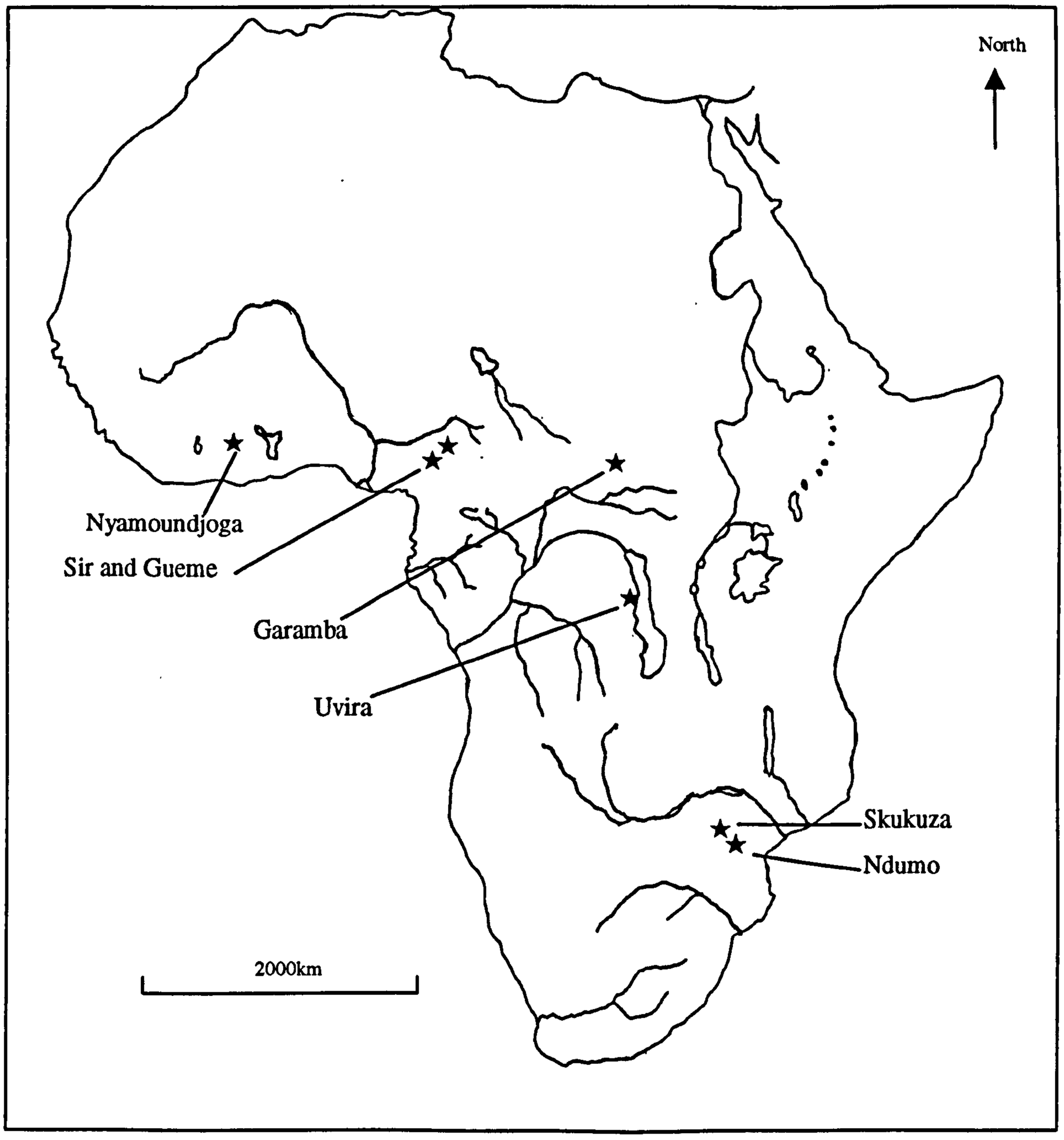


Figure 6-1

Map of Africa showing localities from which samples were collected for the current study.

Snout-vent measures were recorded for each specimen. Incorporation into the study was conditional upon them being sexually mature. Methods for clearing and staining specimens followed those outlined in chapter 3. Osteological characters (listed in Appendix 1, chapter 3) were measured and assessed for variation, and osteometric data analyzed following methods outlined in chapter 3 (univariate analyses, PCA and DFA) and chapter 4 (PCO).

RESULTS

Snout-vent length was compared between sexes for each sample and was significantly greater for females. The magnitude of this variation differed between samples, and was greatest for specimens from Ndumo. It should be borne in mind however, that these differences might be an artifact of small sample sizes (Table 6-1).

Locality	Sex	Sample size	Mean snout-vent (mm)	±Standard Deviation	% difference in mean s-v length between sexes
Ndumo	Male	8	52.36	3.5	22
	Female	10	64	7.0	
Skukuza	Male	6	49.3	1.0	14
	Female	6	56	1.5	
Uvira	Male	8	55.5	3.0	22
	Female	9	67.6	5.0	
Garamba	Male	8	49.83	4.0	9
	Female	8	54.56	4.0	
West	Male	8	50.31	3.5	17
	Female	13	58.92	4.0	

Table 6-1

Sexual dimorphism in snout-vent length (mm) between geographic groups of *X. muelleri*.

Analysis of variance showed mean snout-vent length to be significantly greater in the Uvira sample than in Skukuza, Garamba and pooled western samples ($p=0.000$; d.f=4, 79; $F=5.82$). Other geographic groups did not differ significantly from one another. Males showed the same relative pattern of variation in snout-vent length between localities as did pooled-sex samples ($p=0.007$; d.f.=4,33; $F= 4.24$). Snout-vent length in females was more variable, being significantly greater for Ndumo and Uvira samples than others ($p=0.000$; d.f.=4,41; $F=9.83$) which did not differ significantly from one another.

Osteometric data were analyzed using univariate (continuous measures standardized against snout-vent length) and multivariate (data $\log_e$-transformed) statistical methods for the determination of the phenetic distinctiveness of each population and to identify characters that best accounted for

this variation in order to evaluate their taxonomic significance. Sexes were pooled for analysis owing to sample size restrictions, but were identified in PCO scatter graphs and dendrograms in the final analysis in order to assess the significance of sexual dimorphism within the context of overall osteological variation.

Table 6-2 lists results from one-way analysis of variance that compared data for pooled eastern *X. muelleri* (Ndumo and Skukuza) with that of *X. muelleri* from western Africa (Sir, Namoundjoga, Gueme). Preliminary results indicated that osteology varied considerably between these samples. Characters showing significant variation between geographical groups are listed below (Table 6-2). Means for character values (mm), standardized against snout-vent length (except tooth-count and coracoid/zonal-plane angle), are given for each sample and provide an indication of the form and magnitude of this variation.

Charact-er number	Character	*p≤0.05, **p≤0.01, ***p≤0.001	F value	Mean east	Mean west
2	Skull length	*	6.79	3	2.9
3	Width nasal	***	15.33	0.7	0.8
5	Length nasal	***	20.25	0.68	0.61
10	Lateral septmaxillary / lateral nasal gap	**	8.11	0.2	0.18
11	Lateral septmaxillary/ maxillary arch gap	***	9.25	0.13	0.1
12	Anterior septmaxillary/ nasal gap	***	28.86	0.22	0.15
17b	Frontoparietal/foramen magnum gap	**	8.63	2.4	2.2
20	Length frontoparietal anterior from occipitopetrosal	**	7.55	1.5	1.4
22	Length frontoparietal anterior from pineal foramen	***	97.55	0.8	0.6
23	Distance between level of pineal foramen and anterolateral alae of frontoparietal	***	72.17	0.5	0.3
24	Length frontoparietal posterior from pineal foramen	***	12.86	1.4	1.55
36	Anterior width sphenethmoid	*	8.82	0.7	0.8
39	Length optic foramen	*	5.16	0.13	0.11
40	Retractor bulbi muscle scars	***	17.94	0.09	0.14
41	Anterior width columella	*	5.41	0.08	0.1
43	Length squamosal arm	**	9.37	0.4	0.34
47	Length zygomatic ramus squamosal	*	4.78	0.19	0.17
50	Length premaxillary	**	9.06	0.38	0.34
53a	Length maxillary arch to level of teeth	**	11.21	1.3	1.23
53b	Total length maxillary arch	**	10.29	1.4	1.24
54	Number of pre-maxillary teeth	***	21.53	7.5	6.3
56	Maximum width facial flange	*	5.71	0.047	0.06
62	Distance between otic plates	***	13.3	0.98	0.82
66	Maximum width orbit	**	7.73	0.69	0.64
72	Depth atlas	**	5.58	0.4	0.38
73	Length vertebral column	*	5.33	5.9	6.2
74	Length transverse process I	*	4.22	1.9	1.8
76	Length transverse process III	*	3.24	2.4	2.5
77a	Length sacral diapophysis	***	17.6	2	2.3
77b	Width sacral diapophysis	*	4.74	1.7	1.9

85	Length metatarsal II	**	11.39	4.5	4.2
86	Length humerus	***	14.19	2.1	1.9
87	Length radio-ulnare	**	12.8	1.6	1.5
88	Length metacarpal II	**	12.58	2.3	2.1
89	Length iliac shaft	**	9.84	3.8	4
94	Medial width clavicle	**	6.34	0.3	0.24
96	Length along posterior margin scapula	*	5.22	0.5	0.47
98	Coracoid/ zonal plane angle	***	23.17	72	64
100	Length distal prehallux	**	11.64	0.34	0.31
102	Length ultimate phalanx, metatarsal IV	***	36.95	0.45	0.38

Table 6-2

Results from one-way Anova comparing characters listed in Appendix 1, chapter 3. between pooled eastern *X. muelleri* (Ndumo and Skukuza) and *X. muelleri* from western Africa (Sir, Namoundjoga, Gueme). Asterisks refer to variation significance levels, and F values indicate the relative magnitude of this variation. Degrees of freedom were 1 and 49.

In order to determine how reliably pooled eastern (Ndumo and Skukuza) and western (Sir, Namoundjoga, Gueme) *X. muelleri* could be distinguished apart on the basis of osteometric variation, discriminant function analysis (DFA) was carried out (grouping variable: geographical locality) on $\log_e$-transformed variables. 100% of original cases were correctly classified, corroborating preliminary results from univariate analysis that demonstrated profound osteological variation between groups. Characters of greatest importance (as determined by communality matrix in DFA) for this discrimination were identified by DFA as position of the pineal foramen, nasal proportions and septomaxillary length, number of premaxillary teeth and the angle separating coracoid and zonal axes (Character numbers 22, 23, 102, 12, 98, 54, 5) again corresponding closely with results from univariate analysis. Differences in precise significance levels between results from each of these analyses can be explained in terms of differing effects of standardizing for snout-vent length in the former and for overall size in the latter. Multiple correlations may also affect the outcome in multivariate analysis (Willig, Owen and Colbert, 1986; Corruccini, 1987), and are likely to increase proportionally with the number of variables used. As such, one of the assumptions of DFA is that the ratio of variables-to-repeats be kept to a minimum. Although the degree of similarity between results from multivariate and univariate analyses indicate that the effects of defying this assumption were not significant, PCA was subsequently carried out in order to reduce the number of variables to a small number of linear factors. Each factor represented a linear combination of differentially-weighted variables so as to account for a different orthogonal form of variation. In preceding chapters, this method has enabled the exclusion of a size factor, PCA 1, from subsequent analyses for the purpose of focusing exclusively on shape variation between samples. In the present study however, this was not possible since factor components for PCA1 were not all of high magnitude and positive sign, accounting as much for shape as size variation. DFA was therefore carried out on PCA factors 1-5 (grouping variable: geographic locality), together accounting for only 67% of the total variation in the data. Again, a significant difference was detected between geographical groups (F=117, d.f.=1, 49,

p=0.000), 98% of original cases being correctly classified according to locality. The single mis-assigned individual was from West Africa (Namoundjoga), and was misclassified as Eastern with a probability of 67%. Principal coordinate analysis (PCO) was carried out, using the Euclidean measure of variation, on PCA 1-5. Scatter plots showing phenetic similarity between geographical groups are presented in Figure 6-2 (pooled-sex), and in Figure 6-3 (separate-sex).

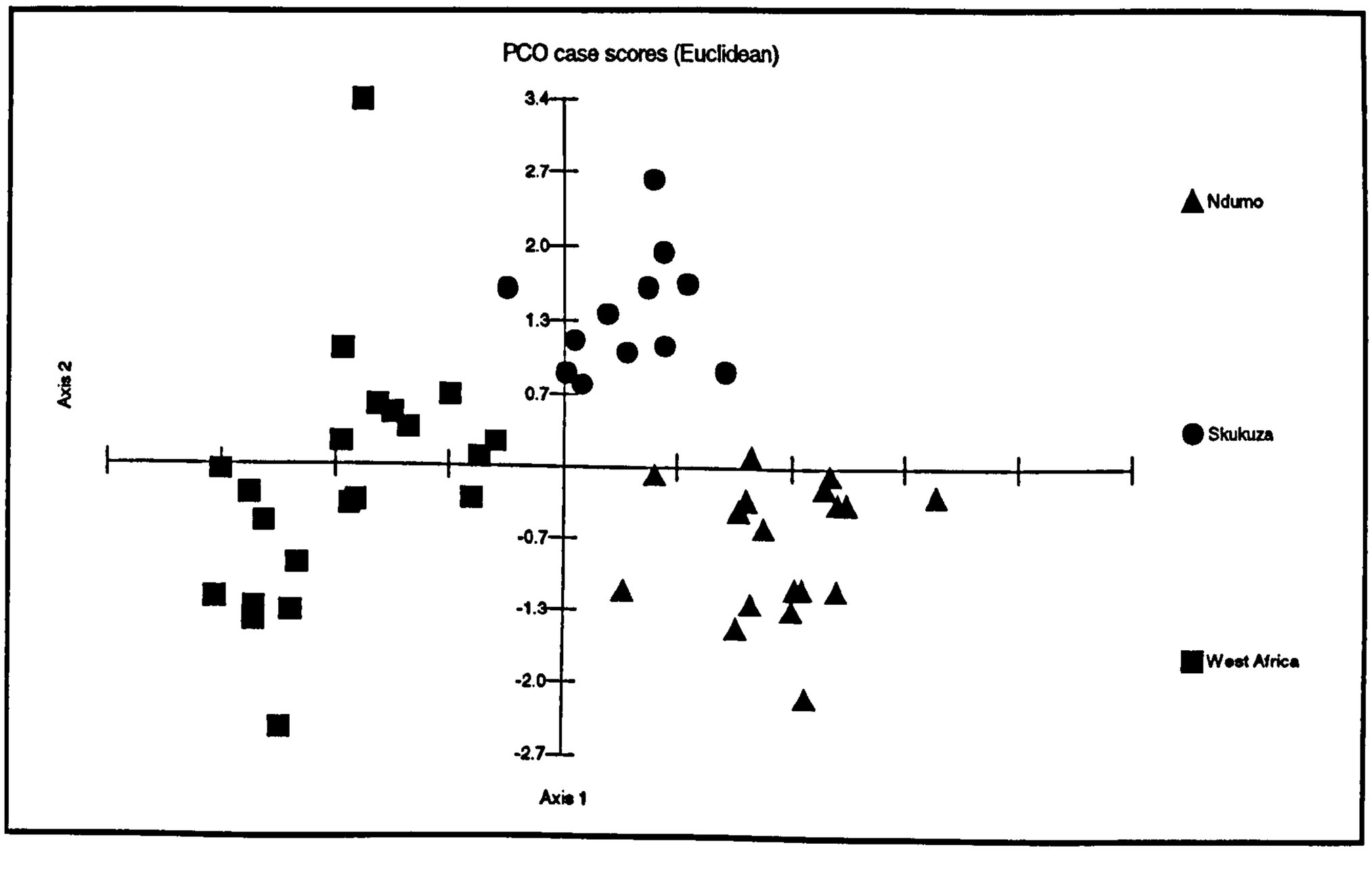


Figure 6-2

PCO (carried out on DFA factors 1-5 [carried out on PCA 1-8)] scatter graph showing phenetic variation between eastern *X. muelleri* (Ndumo and Skukuza) and *X. muelleri* from western Africa (samples from Sir, Namoundjoga and Gueme pooled). Sexes pooled within each geographic group.

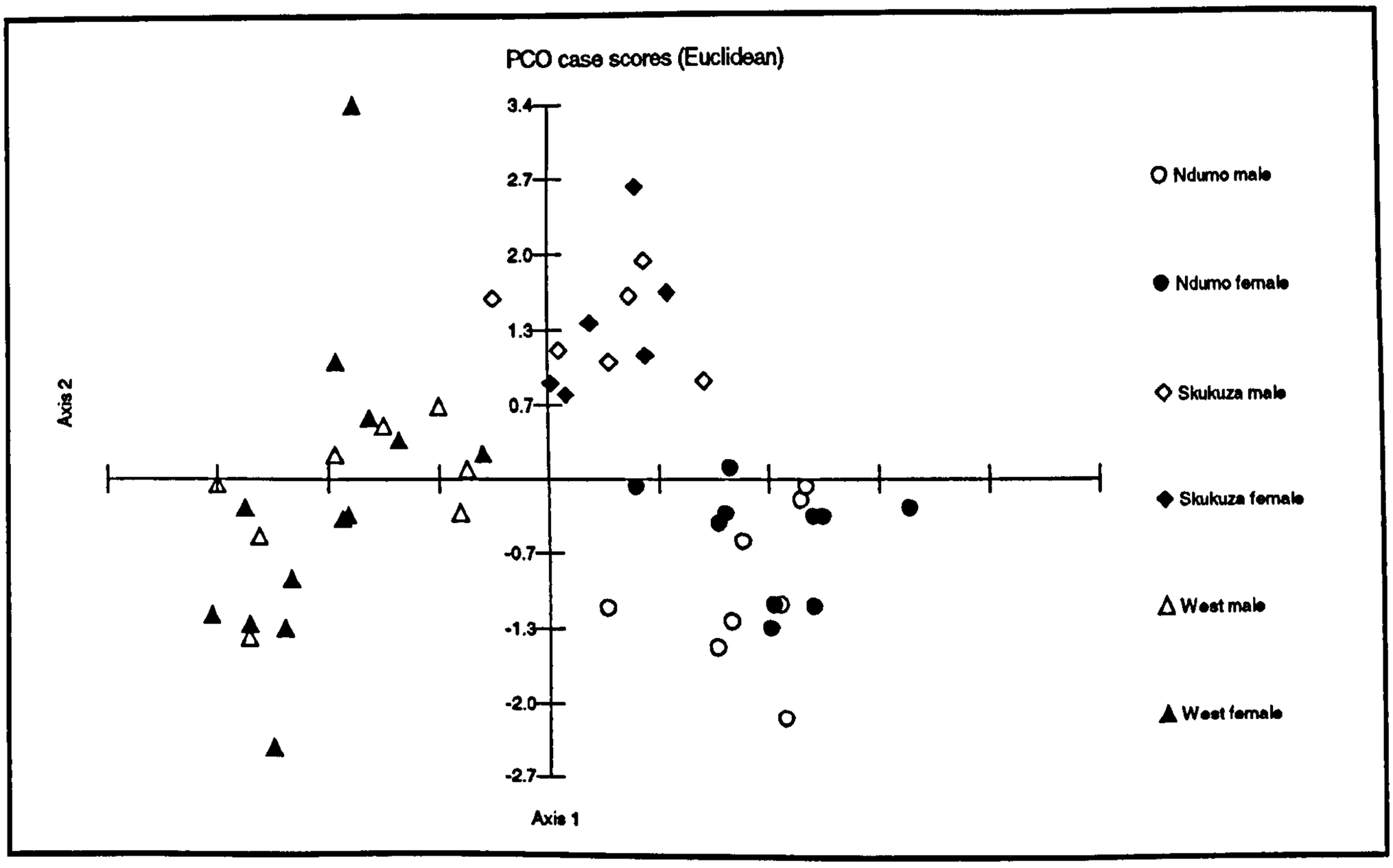


Figure 6-3

PCO (carried out on DFA factors 1-5 [carried out on PCA 1-8)] scatter graph showing phenetic variation between eastern *X. muelleri* (Ndumo and Skukuza) and *X. muelleri* from western Africa (samples from Sir, Namoundjoga and Gueme pooled). Sexes plotted separately for each group.

Given that some information is lost at the level of PCA, PCO results represent a conservative interpretation of phenetic similarity, yet separation between groups remains robust. Axes 1 and 2 account for 70% of the variation explained by PCO (axes 1-3 for 90%) and demonstrate a clear separation between eastern and western groups. This is consistent with results from DFA (both on $\log_e$-transformed data and on PCA factors 1-5). In addition, eastern samples are shown to be phenetically distinct from one another, introducing a further, unexpected phenetic division within *X. muelleri*.

Having established that the osteology of western *X. muelleri* differs fundamentally from that of the eastern form, and that the latter is further divided into osteologically distinct groups which are determined by locality, additional samples collected from intervening areas within the *X. muelleri* range were incorporated into the analysis. Both univariate and multivariate methods were applied to osteometric data, samples distinguished by locality (samples from Sir, Gueme and Namoundjoga were pooled however owing to sample-size restrictions). Each character was analyzed using one-way anova prior to conducting DFA on all $\log_e$-transformed variables. Characters determined by each method to vary significantly between localities are listed below (Table 6-3).

Charac -ter number	Character	**p≤0.01, ***p≤0.00 1	F value	Mean actual length for each of groups 1-5* respectively (cm)
3	Anterior width frontoparietal	***	24.28	.65, .83, .75, .82, .84
5	Length nasal	***	19	.7, .67, .64, .55, .62
10	Lateral nasal/septomaxillary gap	***	10.08	.23, .19, .24, .18, .18
11	Septomaxillary/maxillary lateral gap	***	19.26	.14, .14, .09, .06, .11
12	Anterior septomaxillary/ nasal gap	***	18.94	.24, .19, .21, .14, .15
17b	Length frontoparietal to foramen magnum	***	11.09	2.34,2.41,2.35,2.07, 2.24
22	Length frontoparietal anterior from pineal foramen	***	35.93	.84, .84, .82, .56, .6
23	Length from level of anterior lateral frontoparietal alae to pineal foramen	***	20.39	.49, .53, .47, .34, .31
34	Width vomer		0.7	.51, .50, .55, .49, .51
36	Anterior width sphenethmoid	***	10.51	.76, .7, .68, .73, .79
39	Diameter optic foramen	***	8.81	.15, .12, .09, .13, .12
40	Rtbs	***	8.25	.08, .12, .15, .1, .14
42	Length columella		0.44	.74, .73, .73, .73, .74
43	Length squamosal	***	9.49	.38, .42, .49, .4, .34
44	Length tympanic annulus		1.41	.73, .68, .71, .75, .73
50	Length pre-maxillary	***	10.54	.39, .35, .38, .33, .34
56	Maximum width facial flange	**	3.95	.05, 0.4, .07, .07, 0.6
54	Number of premaxillary teeth	***	11.67	7.39,7.75,8.12,6.19, 6.3
55	Number of maxillary teeth	***	14.43	21, 23, 24, 18, 22
71	Width atlas		0.39	.83, .81, .81, .82, .82
72	Depth atlas	***	8.82	.44, .38, .37, .36, .38
77	Length sacral diapophysis	***	6.84	2, 2.05, 2.25, 2.21, 2.33
83	Length fibio-tibula	*	3.31	4.19, 4.06, 4.04, 3.89, 3.97
84	Length fibiale-tibulare	**	4.93	2.44, 2.3, 2.29, 2.18, 2.28
87	Length radio-ulnare	***	9.55	1.65, 1.57, 1.65, 1.45, 1.49
90	Length ischium	***	10.08	.71, .6, 0.74, .61, .69
91	Width anterior acetabular	***	6.88	1.73, 1.73, 1.74, 1.58, 1.74
92	Width posterior acetabuar	**	4.22	1.03, 1.01, 1.06, 0.96, 1.03
94	Clavicle medial width	***	6.4	.28, .35, .22, .27, .25
95	Distance between medial margins clavicle and coracoid		1.4	1.2, 1.7, 1.5, 2.5, 1.5
96	Length post margin scapula	***	6.49	.52, .48, .44, .52, .47
98	Coracoid/ zonal plane angle	***	10.24	74, 69, 68, 67, 35º
99	Length proximal prehallux		1.07	.21, .19, .32, .21, .19
100	Length distal prehallux	***	8.41	.36, .34, .37, .33, .31
102	Length ultimate phalanx metatarsal IV	**	3.55	.46, .44, .43, .37, .38
103	Length pre-pollex	***	7.88	.13, .13, .1, .12, .13

Table 6-3

* Geographic *X. muelleri* groups 1-5 (right-hand column): Ndumo, Skukuza, Uvira, Garamba and West (pooled Namoundjoga, Sir and Gueme). Characters, standardized against snout-vent length (except for tooth count and angle data), were compared between geographic groups using one-way Anova. Asterisks refer to significance

levels of character variation between groups. Means and F values are given for each sample, providing an indication of the direction and magnitude of variation for each character. Degrees of freedom were 4, 79.

% correct classification of cross validated cases						
Ndumo	100	0	0	0	0	100
Skukuza	0	100	0	0	0	100
Uvira	0	0	100	0	0	100
Garamba	0	0	0	93.75	6.25	100
West	0	0	0	0	100	100
Summary: 98.8% correct classification						

Table 6-4

Correct classification rates for each sample according to geographic locality

All except one of original cases were correctly classified by DFA according to geographical locality and variation between populations was shown to be significant (F=40.52; d.f.=4, 79; p=0.000). The single misclassified case was borderline, 48:52%, a Garamba specimen mistakenly placed in the Western sample. Characters were listed in order of discriminatory importance and given as: 22, 23, 102, 12, 98, 54, 5, 40, 100, 94 (see Appendix 1, chapter 3), which compared well with univariate results (Table 6-3), and were identical to the sequence of characters given by the same analysis when applied to the discrimination between Eastern and Western samples. The form and magnitude of variation of these characters is given in Table 6-3. Although carrying out DFA on all $\log_e$-transformed variables defies the assumption that the number of variables-to-repeats be kept low, results were consistent for each of these statistical methods. The high rate of correct classification not only demonstrated that eastern populations are distinct from those in the west, but also that the Ndumo, Skukuza and Uvira samples are quite distinct from one another. On the whole, Western and Garamba specimens were also phenetically distinct. The total number of variables was subsequently reduced for the purpose of conducting PCO analysis of phenetic similarity between geographical samples. Variables that contributed least to inter-group variation were first selected for removal using DFA and PCA, removing those with highest Wilk's λ and lowest component weightings respectively (These were 1b, 29, 34, 42, 44, 51, 52, 53b, 66, 67, 71, 73, 76, 78, 89, 95, 99, 101- cross reference with Appendix 1, chapter 3). PCA was then carried out on remaining variables in order to reduce data to a small number of factors. PCA1-5 accounted for 70% of the total variation explained by the analysis and was subsequently used in DFA followed by PCO analysis. PCO was carried out on DFA 1-4 (of PCA 1-5) (grouping variable: geographical locality). The first two axes of PCO scatter plots accounted for 71.8% of variation in the data, showing each of the eastern populations to be distinct from one another, and in turn distinct from a cluster representing samples from Garamba and west Africa. The Garamba sample is partially separated from other western specimens along a third axis, not shown here in order to preserve clarity. No separation of each of the three, pooled western

samples is evident. Furthermore, the direction of variation demonstrated by scatter plots (Figure 6-4 and Figure 6-5) indicates that variation among eastern samples is in a different direction to that which separates western and Garamba samples. Figure 6-4 shows sexes pooled by group. In Figure 6-5 sexes are plotted separately for each group.

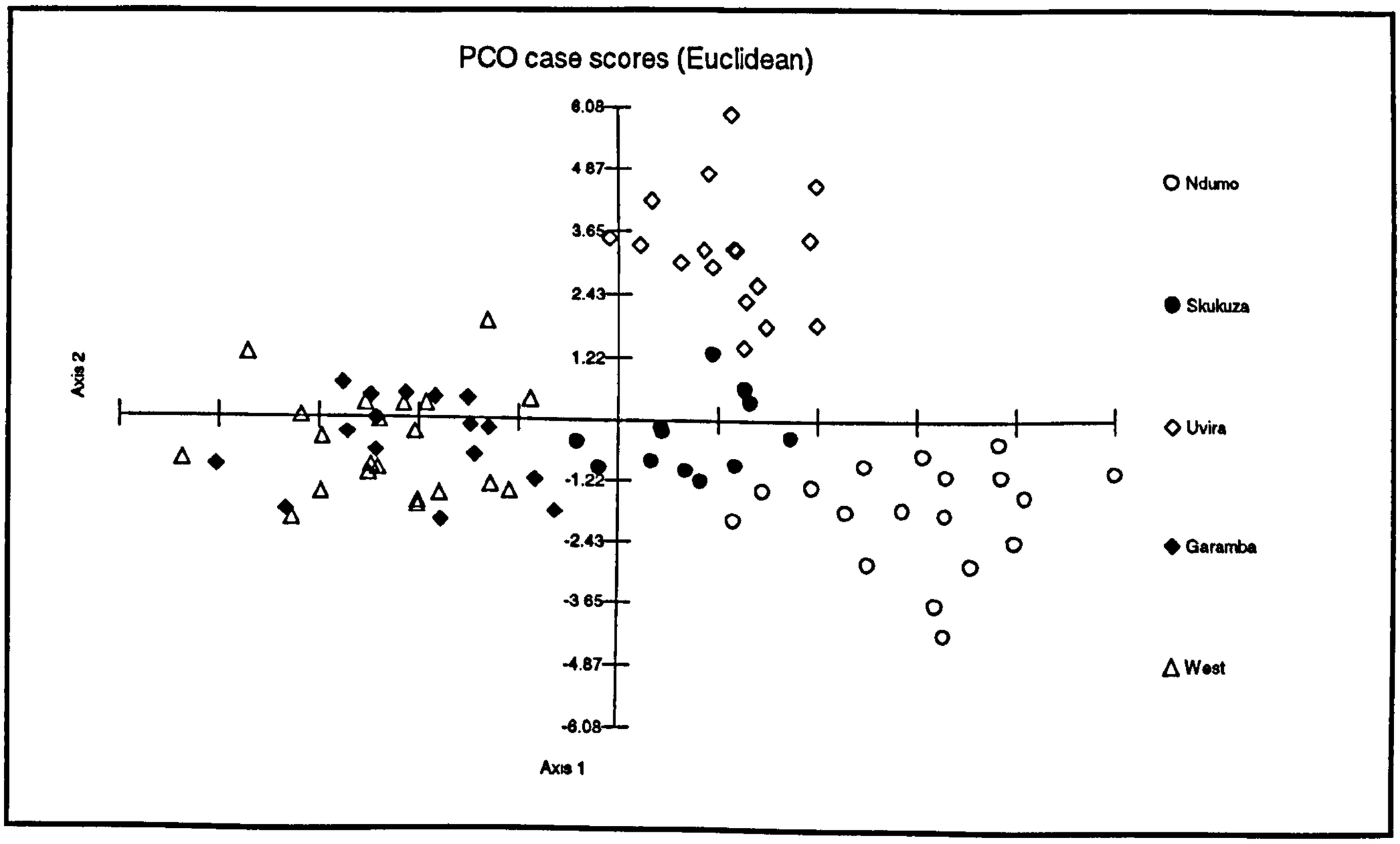


Figure 6-4

PCO (carried out on DFA factors 1-4 [carried out on PCA 1-5)] scatter graph showing phenetic variation between geographic *X. muelleri* groups: Ndumo, Skukuza, Uvira, Garamba and western Africa (pooled Sir, Namoundjoga and Gueme). Sexes pooled by group.

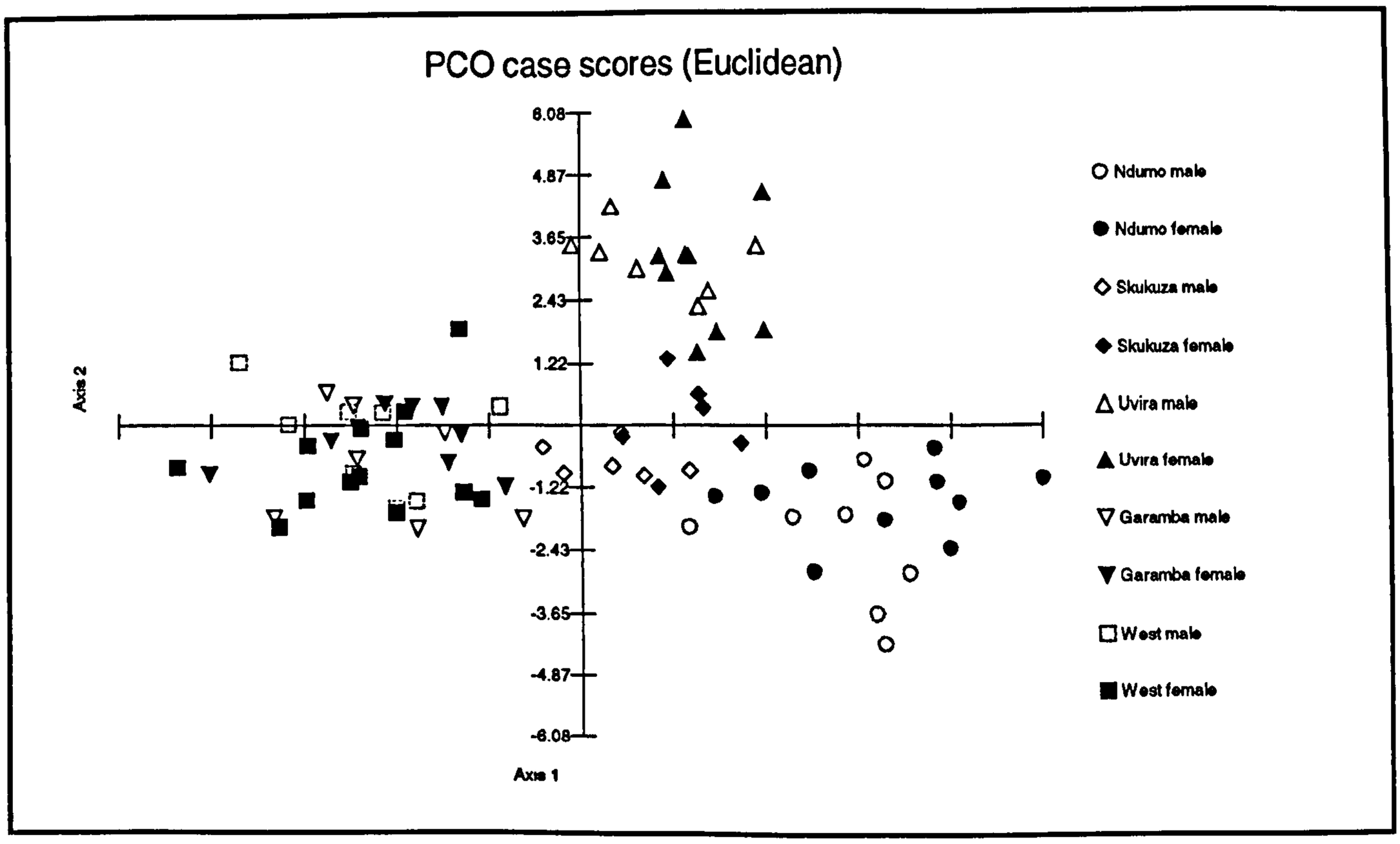


Figure 6-5

PCO (carried out on DFA factors 1-4 [carried out on PCA 1-5)] scatter graph showing phenetic variation between geographic *X. muelleri* groups: Ndumo, Skukuza, Uvira, Garamba and western Africa (pooled Sir, Namoundjoga and Gueme). Sexes plotted separately for each group.

Scatter plots show sexes to overlap widely within each geographical group, demonstrating that sexual dimorphism is not significant within the context of overall phenetic variation. Results are consistent in showing that *X. muelleri* does not form a polytypic cline across the lowland savanna range defined above, but rather, demonstrates a far more complicated pattern of variation. This pattern, albeit complex in the case of samples from Uvira and East African samples, is consistent with there being discrete eastern and western *X. muelleri*-forms, each comprising phenotypically distinct sub-groups.

Results from a cluster analysis (UPGMA) of DFA 1-4 (of PCA 1-5, grouping variable: geographic locality) are presented in the form of a dendrogram (Figure 6-6). This analysis also demonstrates robust separation between eastern and western groups. Within the 'western' cluster, Garamba specimens are distinct from pooled western samples (pooled samples not differentiated according to locality), and within the 'eastern' cluster, each of Uvira, Skukuza and Ndumo samples are separate, the Uvira sample shown to be a sibling to nested Skukuza and Ndumo samples. Sexes do not form sub-groups within each geographical cluster, again indicating that within the context of overall phenetic variation, sexual dimorphism is not significant. The branch connecting a single specimen from West Africa is particularly short; cross-reference to probability of group membership

values (DFA) showed this specimen to have a 38% probability of belonging to the Skukuza sample, whilst its probability of belonging to the western sample is 60%.

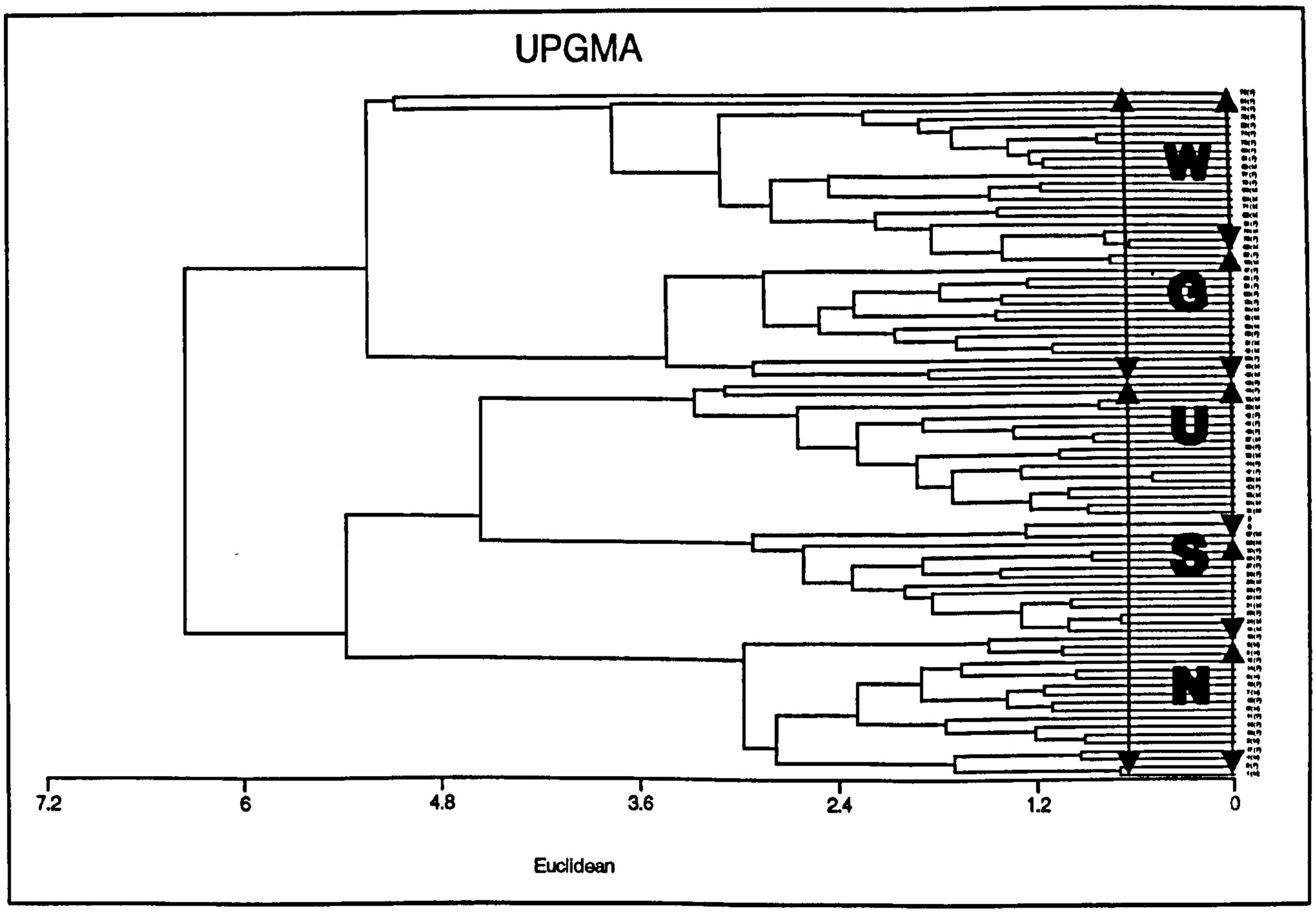


Figure 6-6

UPGMA cluster analysis (using MVSP) of osteometric data for *X. muelleri* from each of 7 localities: Western Africa (Pooled Sir, Gueme and Namoundjoga), Garamba, Uvira, Skukuza and Ndumo. Branching order (horizontal) and length correspond to phentic similarity between geographical groups. Each locality is delimited by a short vertical arrow, adjacent to which is a letter corresponding to the first letter of each locality name. Longer vertical arrows delimit eastern and western clusters. Sexes are not nested within geographic locality.

DFA was carried out in order to identify the most discriminatory characters that distinguish between samples from Uvira, Skukuza and Ndumo. Results are presented in Table 6-5, and should be cross-referenced to mean character values in Table 6-3 in order to ascertain the form and degree of variation between groups. Similarly, characters that distinguish Garamba from western African samples are presented in Table 6-6.

Ndumo/ Skukuza. F=47, d.f.=1, 28, p=0.000		Ndumo/ Uvira. F=30, d.f.=1, 30, p=0.000		Skukuza/ Uvira. F=52, d.f.=1, 27, p=0.000	
Character number	Character	Character number	Character	Character number	Character
50	Premaxillary length	11	Lateral distance between septmax/maxillary	50	Premaxillary length
96	Scapula length	95	Medial distance clav/cor	100	Distal prehallux length
39	Diameter optic foramen	39	Length optic foramen	71	Width atlas
12	Anterior septmax/ nasal gap	40	Distance between rtbs scars	44	Tympanic annulus length
72	Depth atlas	103	Prepollex	10	Lateral septmax/ lateral nasal gap
84	Length fibiale-tibulare	3	Maximum width nasal	87	Radio-ulnare length
10	Lateral septmax/ lateral nasal gap	55	Number maxillary teeth	56	Facial flange maxillary width
95	Distance between medial margins clavicle/coracoid	98	Coracoid- zonal plane angle	90	Length ischium
5	Length nasal	100	Distal prehallux length	34	Maximum width vomer
90	Length ischium	43	Length zygomatic process	42	Length columella
36	Anterior width shenethmoid	94	Medial width clavicle	77	Length sacral diapophyses
99	Length proximal prehallux	34	Maximum width vomer	92	Caudal width acetabular
87	Length radio-ulnare	96	Scapula length	84	Fibiale-tibulare length
83	Length fibio-tibula	21	Gap between frontoparietal and foramen magnum	91	Rostral width acetabular

Table 6-5

Results from DFA to identify the strongest discriminatory osteological characters between paired geographic groups of *X. muelleri*. Characters are sequenced in order of discriminatory importance. P values indicate the level of significance of variation between groups, whilst F values indicate the magnitude of this variation. Character numbers may be cross-referenced to those listed in Appendix 1, chapter 3. Abbreviations, rtbs: retractor bulbi muscle scars.

Garamba/ West Africa. F=38, d.f.=1, 35, p=0.000	
Character number	Character
21	Length frontparietal posterior to point of association with occipitopetrosal
5	Length nasal
17a	Length frontoparietal
91	Anterior width acetabular
41	Width columella
55	Number of maxillary teeth
11	Distance between lateral margin septomaxillary and maxillary arch
65	Width skull at level of maxillary/pterygoid intersect
31	Distance between medial margins of ventral occipitopetrosal margins
2	Length skull
30	Foramen magnum depth
17b	Distance between frontoparietal and foramen magnum
57	Medial depth pars palatina
75	Length second transverse process

Table 6-6

Results from DFA to identify the strongest discriminatory osteological characters between Garamba and western African samples of *X. muelleri*. Characters are sequenced in order of discriminatory importance. P values indicate the level of significance of variation between groups, whilst F values indicate the magnitude of this variation. Character numbers may be cross-referenced to those listed in Appendix 1, chapter 3.

Xenopus muelleri (west)- Osteological Description

Cranium

Cranium length and width are approximately equal, and the three main components are moderately narrow.

Frontoparietal

This parallel-sided or hourglass-shaped element is as wide anterolaterally as it is at its anterior-most point of association with the occipitopetrosal (Illustration 6-1). The generally rounded posterior profile bears a pair of posterolaterally directed alae. The apex is square-angled or acute and partially overlies the posterior nasal margin; margins may be slightly concave with respect to one another and extend towards pronounced anterolateral corners of the main element body. Parasagittal crests are widely separated and diverge anteriorly. Posteriorly, crests converge, their margins forming a broad, U-shaped profile within the posterior margin of the element. The skull table, as delineated by these crests is very flat, and is almost as wide as the element itself, giving rise to very steeply sloped lateral cranial walls that associate with, and are occasionally fused to, the sphenethmoid. The pineal foramen is positioned within the anterior-most third of the element, adjacent to or just posterior to anterolateral corners of the element.

Sphenethmoid and vomer

This element is either parallel-sided or slightly waisted; in the latter case, antero- and posterolateral widths are approximately equal (Illustration 6-2). Laterally-displaced, paired notches occur along the anterior margin and represent orbitonasal foramina. Retractor bulbi muscle scars (rtbs) are separated by a gap that varies between being very narrow to one that measures approximately one-third the width of the element, the latter being the more common state. Optic foramina are usually small, partially visible in ventral view and occur within rtbs. Posterolateral alae are short, under-developed and are considerably narrower than the main body of the element. The caudal process may be either parallel-sided or bulbous in shape and does not reach quite as far as the foramen magnum. The degree of fusion between sphenethmoid and occipitopetrosals in this region is high, often making the determination of the shape of this caudal process impossible. The azygous vomer is large and toothed with convex anterior and posterior margins that narrow to a point laterally.

Occipitopetrosal

Anterior and posterior margins are oriented obliquely, converging on moderately well-developed cristae parotica (Illustration 6-1, Illustration 6-2). Paired elements are medially fused around the foramen magnum. Dorsal crests are well-developed, giving the element the appearance of

bi-lateral concavity. Eustachian troughs are deep, and the ridges that border their posterior margins extend medially towards the widest part of the caudal sphenethmoid process. The prominences that extend from mid-way along these ridges vary in size and robustness from barely breaking the surface of the convex auditory capsules to providing a large and prominent surface for the attachment of cervical muscles.

Pterygoid

The deep proximal flange of the anterior pterygoid ramus is usually, but not always, anterolaterally cleft, and the distal flange only moderately curved. The lateral ramus lacks any distinguishing features. Otic plates however, are characterized by their small size and wide inter-otic gap. They are approximately symmetrically bifid, the anterolaterally directed ala extending to meet posterolateral alae of the sphenethmoid, whilst the other extends medially, covers only a small proportion of the Eustachian trough and has an obliquely oriented posterior margin.

Tympanosquamosal and columella

The annulus is round to sub-oval and lacks distinct otic proceses to support the columella at its pivot point (Illustration 6-1). A semi-robust squamosal extends from the anterodorsal annulus margin to overlap and associate dorsally with the distal flange of the anterior pterygoid ramus. Despite lacking a distinct zygomatic ramus, the squamosal is distally expanded in its region of association with the pterygoid. The junction formed between annulus and squamosal varies between being deeply cleft and U-shaped. A short, robust stalk forms the medial annulus base. Extending from the medial margin of this stalk is a short process that overlaps the crista parotica. The columella is widest at its base, the pars interna plectra, narrowing along the pars medial plectra, widening slightly at its pivot point with the tympanum.

Maxillary arch

A long, square-ended maxillary arch reaches half way across the orbit and beyond the anterior pterygoid ramus (Illustration 6-2). The dental row extends the full length of pre-maxillaries and along approximately three quarters the length of maxillaries. Teeth are small, and partes facialis and palatina are widest medially, diminishing in width gradually, the former reaching no further than the level of the anterior orbit, the latter, the end of the dental row. A small degree of overlap occurs between the pars facialis and alary process.

Mandible

As for account in chapter 2 (Illustration 6-3).

Nasal and septomaxillary

The azygous nasal is neither medially scarred nor anteriorly cleft (Illustration 6-1). The anterior ramus is parallel-sided and distal curvature is moderate. Anterior margins of lateral rami are

almost always perpendicular with respect to the rostral process, but may otherwise be sub-perpendicular. Septomaxillaries are long, lack lateral foramina and are oriented at a low angle with respect to that of the body.

Calcification

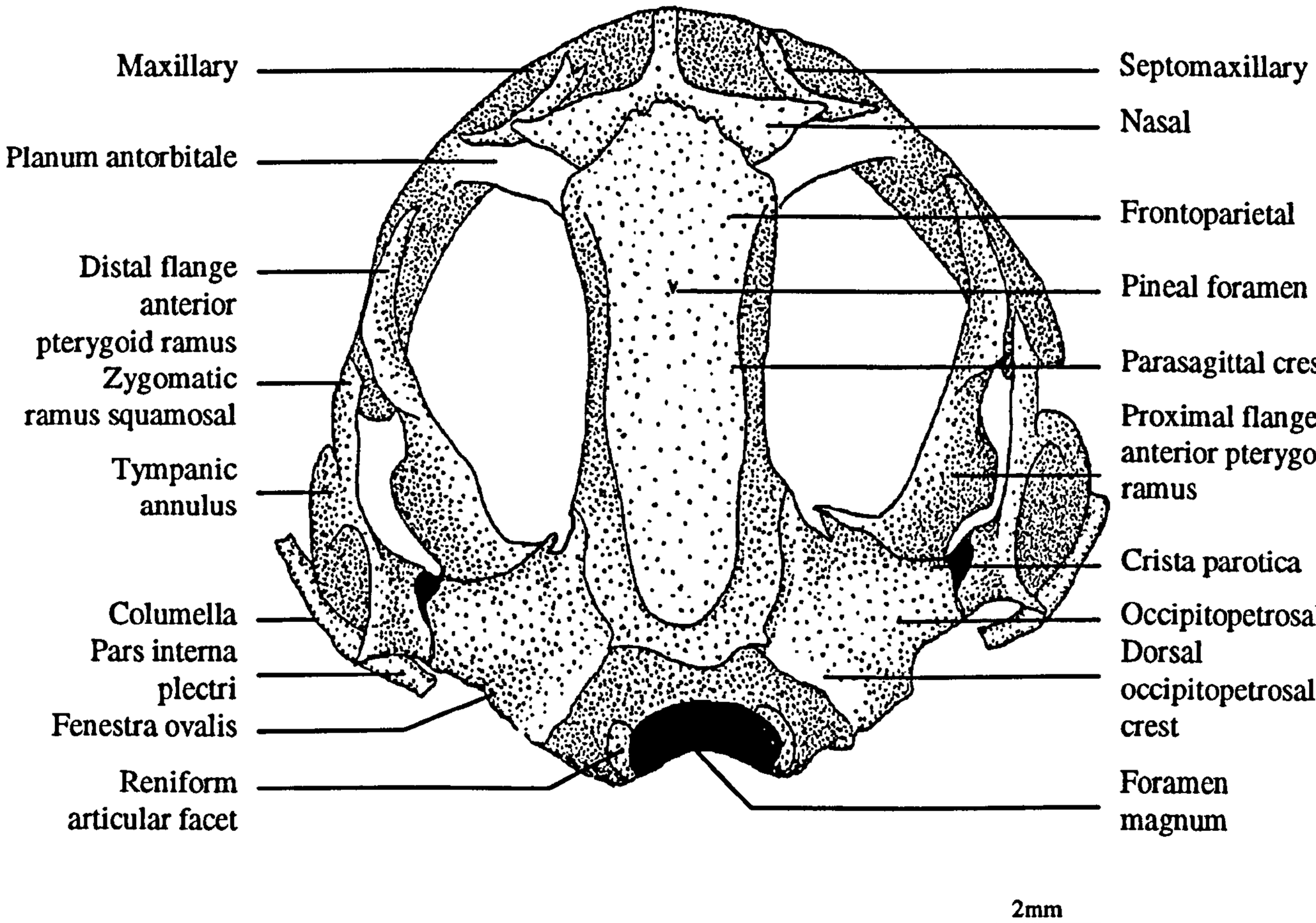
There is a high degree of calcification of rostral cartilaginous elements. However, some elements remain un-calcified regardless of the extent of calcification in others. Most specimens exhibit calcification of the planum antorbitale, cartilage surrounding the distal tip of the rostral nasal extension and the septum nasi. In some, the cartilage associated with alary processes is also calcified. However, element A (*sensu* Smirnov, 1994) remained un-calcified in all specimens examined. Elements are listed in order of the prevalence of calcification: planum antorbitale, nasal rostral and septum nasi, alary process.

Degree of fusion

Fusion between adjacent elements is extensive, occurring between all cranial elements except for the vomer and sphenethmoid, which remain distinct in all specimens examined.

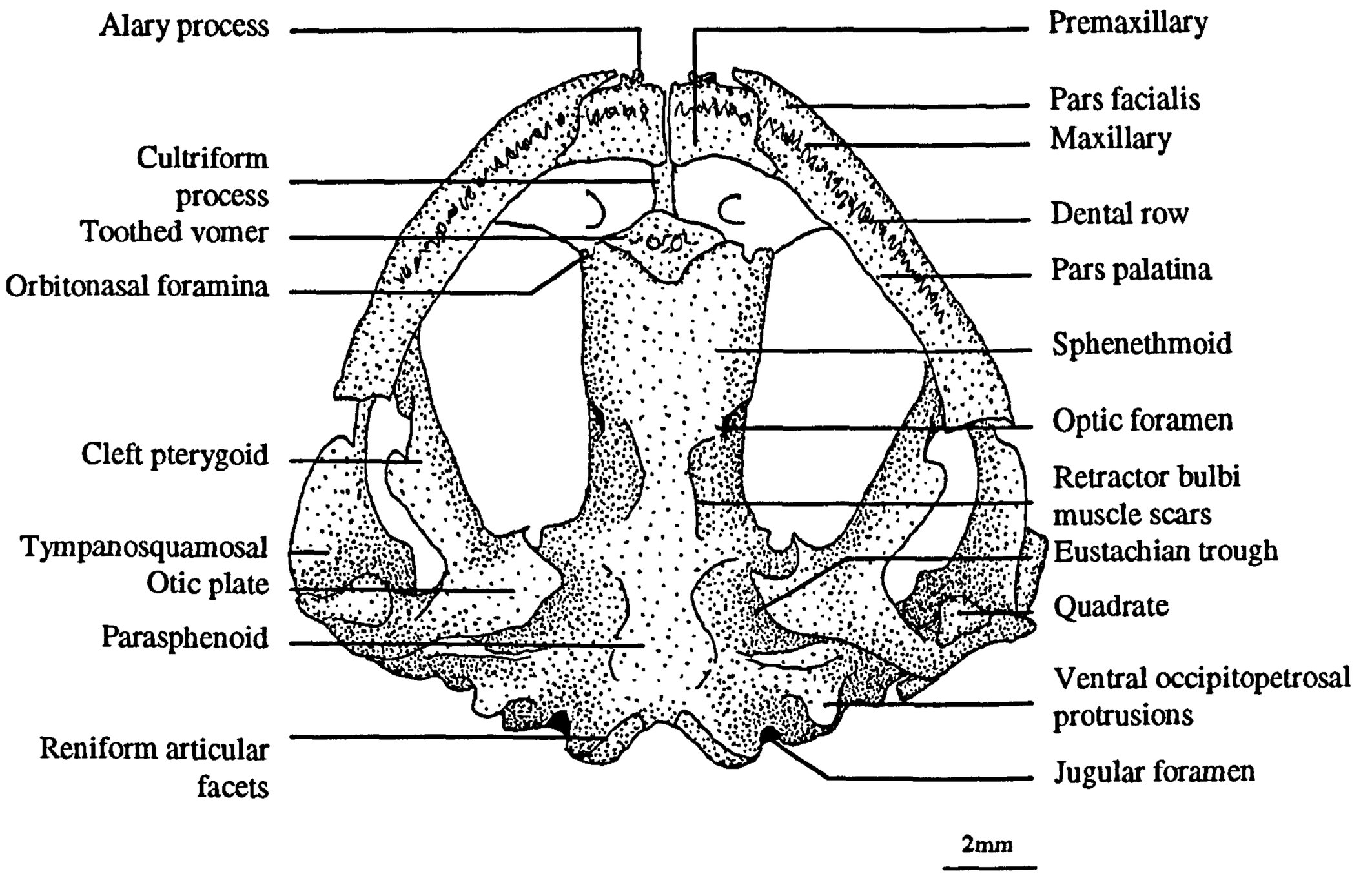
Hyolaryngeal apparatus

As for account given in chapter 2.



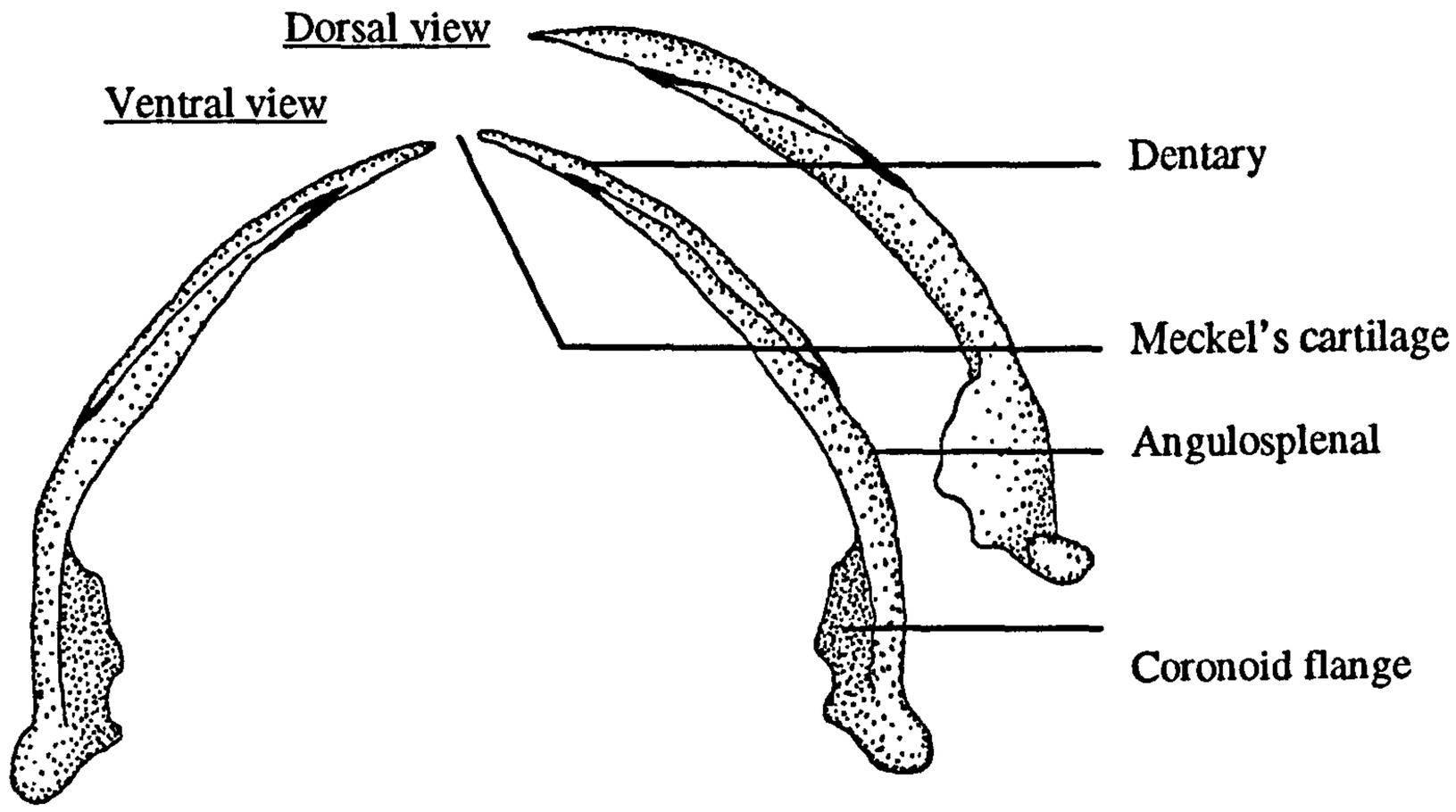
Dorsal view *X. muelleri* (west) skull

Illustration 6-1



Ventral view *X. muelleri* (west) skull

Illustration 6-2



X. muelleri (west) mandible

Illustration 6-3

Axial skeleton

The vertebral column is long and narrow, as determined by relative width of vertebrae, transverse processes and sacral diapophyses (Illustration 6-4). Pre- and post-sacral portions are approximately equal in length. The atlas assumes a triangular dorsal profile and bears a concave anterodorsal profile with widely spaced atlantal cotyles and narrows significantly towards its posterior margin, which bears paired dorsal spines. All subsequent vertebrae, except for the ultimate pre-sacral, bear only a single dorsal spine and are moderately well ornamented. The ultimate pre-sacral vertebra occasionally bears a discontinuous posterior margin.

The first pair of transverse processes is oriented either at right angles or slightly anterolaterally on leaving the vertebral column. Processes are generally narrow proximally, widening towards their mid-point before tapering distally, each process measuring approximately the same in length as the atlas in width. The second pair of transverse processes are the longest of all, measuring approximately 150% that of the first pair and the atlas. These are oriented anterolaterally on leaving the vertebral column and curve posterolaterally. Curvature may either occur proximally or evenly along the entire length of the element. The third pair is considerably more curved than the anteriorly adjacent pair. Similarly they are anterolaterally orientated on leaving the vertebral column and curve posterolaterally. Curvature varies from being quite pronounced half way along the process to being even along its entire length (the greatest curvature is seen in SR32). The second and third pairs of transverse processes are of equal widths, both bearing terminal expansions and cartilaginous caps, those of the third pair being largest. Ventrally, the atlas bears a convex, medially notched anterior margin. Articular faces between centra are generally very wide, though there are exceptions to this. Correspondingly, spinal foramina separating pedicels that form the base of each transverse process are small. Sacral diapophyses in this sample are almost all asymmetrical around the transverse plane, but variation in the degree of asymmetry accounts for much of the total variation seen in the axial skeleton of this sample. Although generally parallel-sided, their margins may occasionally diverge or converge anteriorly. There is a high occurrence of vestigial processes within the sample. Osteological deformities include fusion of vestigial process to the sacrum and the occurrence of twin dorsal spines on the atlas of one specimen and on vertebra III and IV of another.

Anterior appendicular skeleton

Pectoral girdle

Suprascapula and cleithrum are short and are separated mid-dorsally by a considerable gap (Illustration 6-5). They articulate laterally with the scapula. A deep, narrow cleft separates partes acromialis and glenoidialis of the scapula. Clavicles are equal in width along their entire length and gently arch anteromedially. Elements are medially separated by a narrow gap. Medial clavicle

margins in one specimen are slightly expanded. Medial coracoid widths vary from being equal to measuring approximately one and a half times the lateral width of the element. Their medial margins are separated by a gap that measures the same as the distal width of the element, demonstrating the same range of variation. The mean angle formed between the main zonal plane and coracoid is high. The prevalence of calcification of epicoracoid, suprascapular and sternal cartilage in this sample is nil.

Forelimb

As for account given in chapter 2. The only specific feature is the particularly long phalanges.

Posterior appendicular skeleton

Pelvic girdle

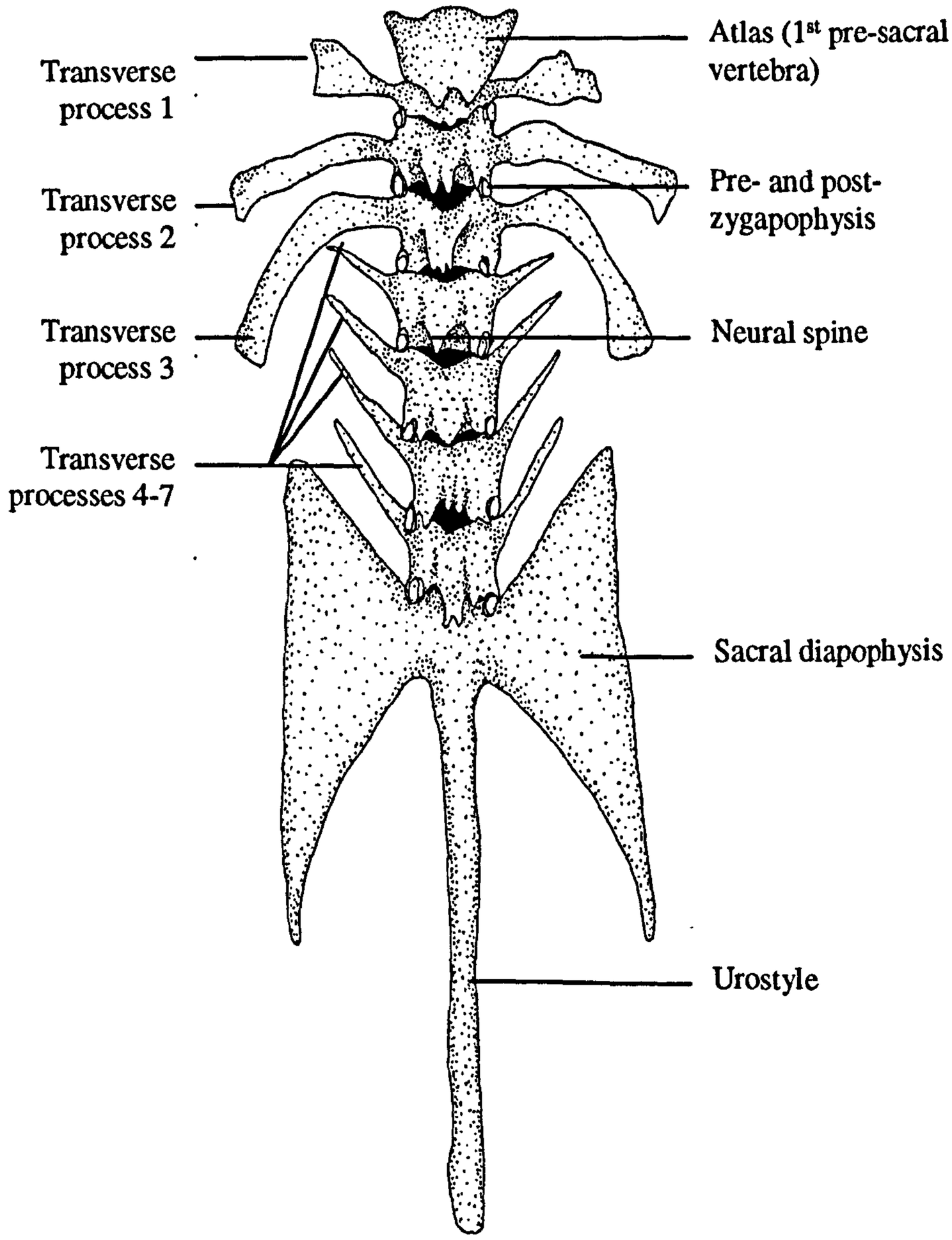
Iliac processes are either parallel-sided or diverge slightly. Dorsolateral crests are prominent and converge to form a wide U-shaped inter-iliac symphysis. Flanking the symphysis are large, paired, dorsal prominences. All pelvic elements are fully ossified and synostotically fused in the majority of specimens.

Hindlimb

Most of these elements conform to the account given in chapter 2, but a few characters are noteworthy; tibiale and fibulare are fully fused, both proximally and distally. Distal prehallical elements are conical and measure approximately twice the length of the oval, proximal element.

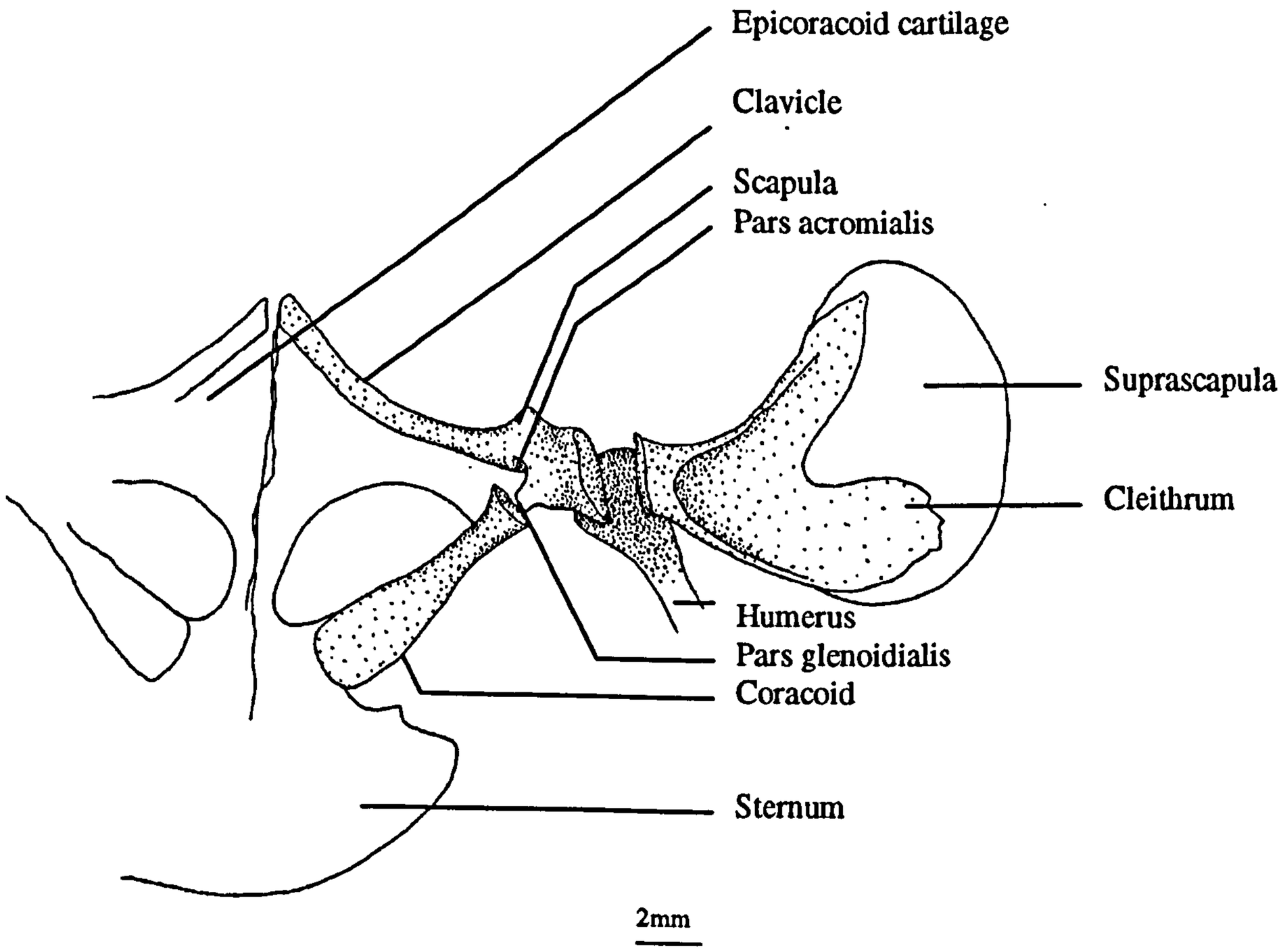
Sexual dimorphism

The tympanic annulus is orientated more dorsally in males and is larger than in females. As a result, the relative length of squamosal is reduced in males. The degree of overlap between the tympanosquamosal base and occipitopetrosals is greater in females.



Dorsal view *X. muelleri* (west) vertebral column

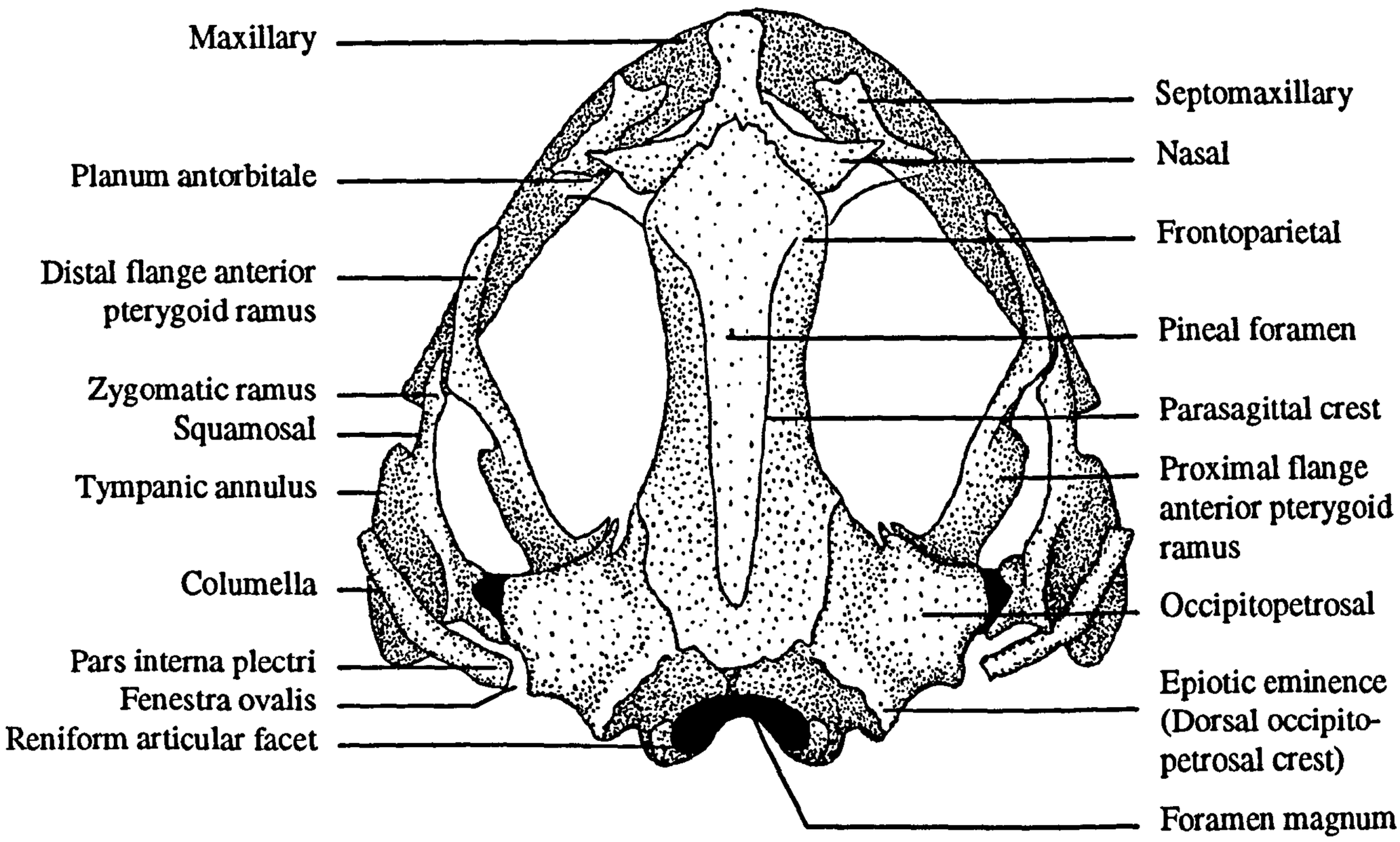
Illustration 6-4



Pectoral girdle *X. muelleri* (west)

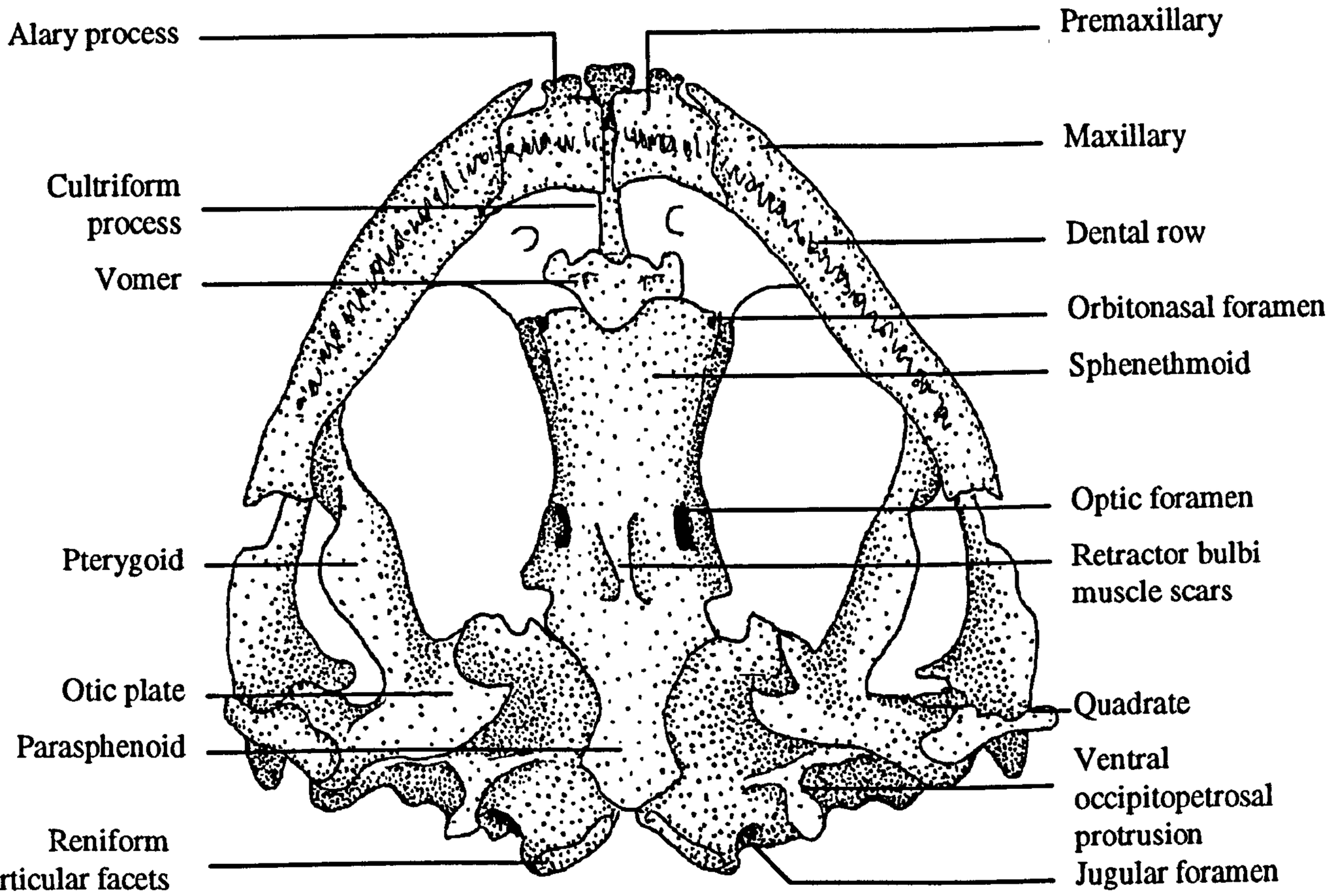
Illustration 6-5

Xenopus muelleri east (Uvira, Skukuza, Ndumo) differs in a number of fundamental respects from *X. muelleri* west (Garamba, Nyamoundjoga, Sir, Gueme) but also demonstrates a significant degree of variation between sites that are only a few kilometers distant from one another. The following illustrations are of a female specimen from Ndumo. Whilst the account which follows these illustrations outlines the principal features that distinguishes *X. muelleri* east from west, it should be borne in mind that the eastern morphotype is highly variable.



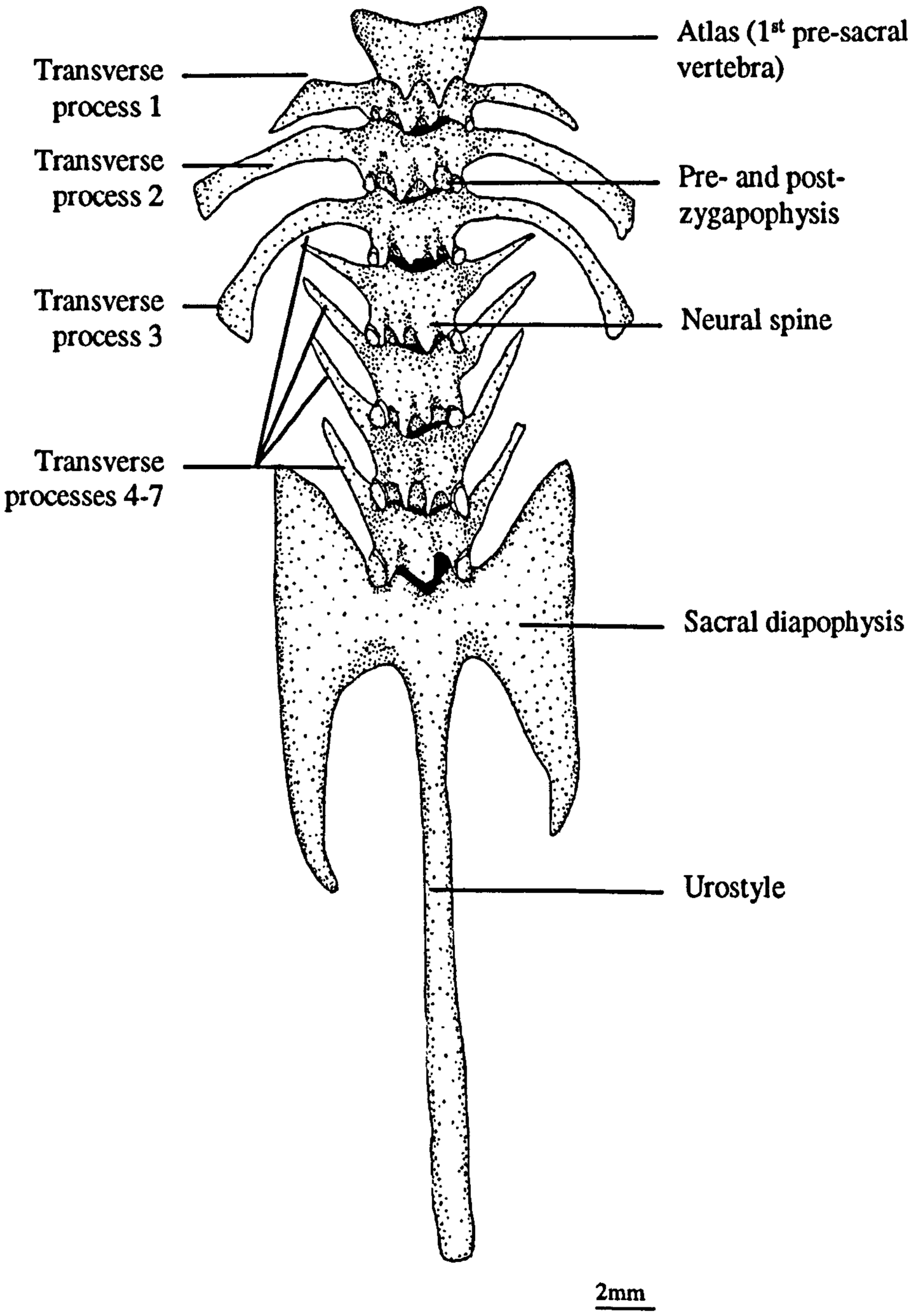
Dorsal view *X. muelleri* (east) skull

Illustration 6-6



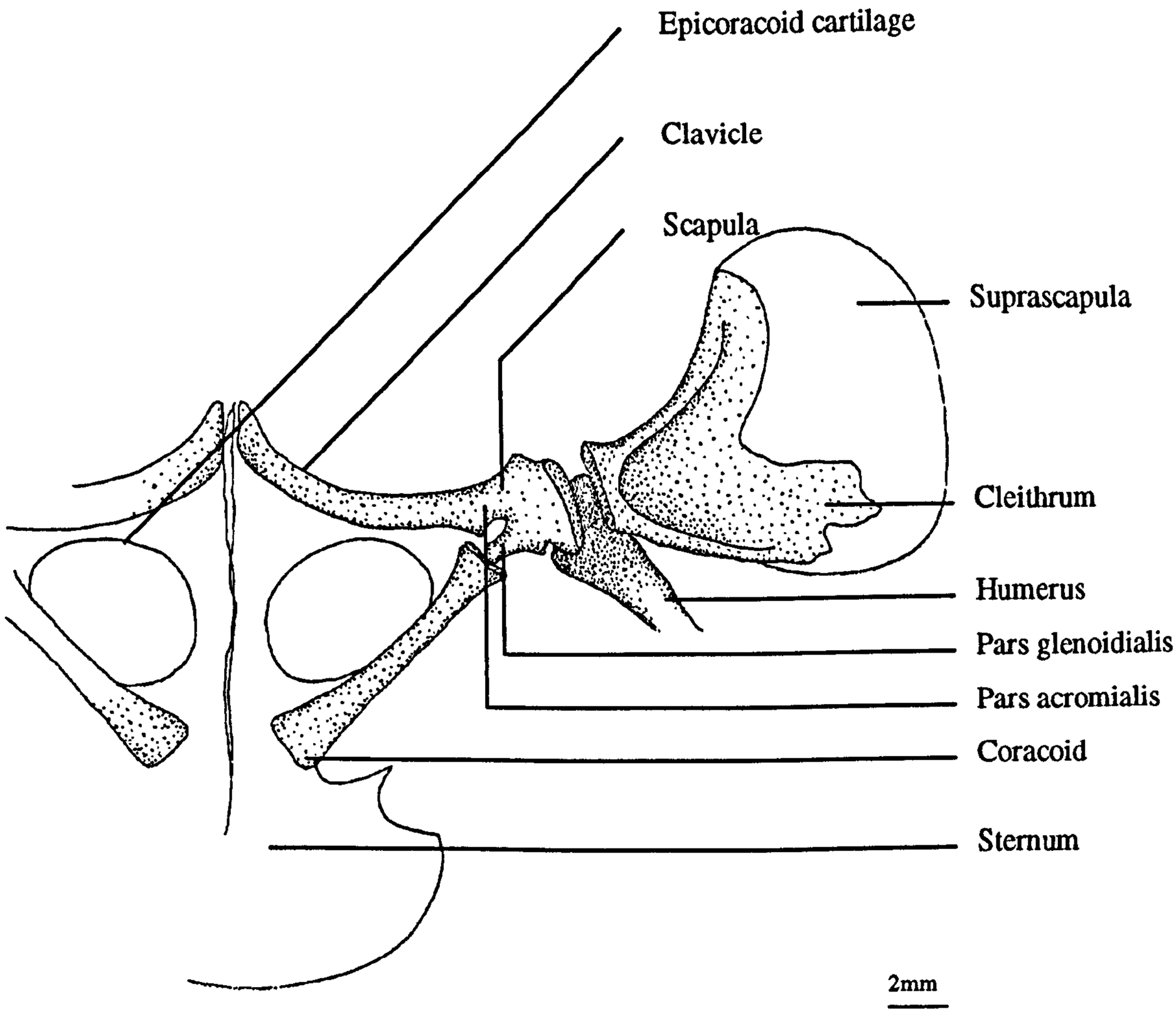
Ventral view *X. muelleri* (east) skull

Illustration 6-7



Dorsal view *X. muelleri* (east) vertebral column

Illustration 6-8



Pectoral girdle *X. muelleri* (east)

Illustration 6-9

Principal characters that distinguish *X. muelleri* east from west

Whilst *X. muelleri* east and west broadly follow the same phenotypic pattern, some fundamental differences distinguish the two apart. Since the eastern form is so variable, no full account has been given, instead the following account has been assembled which draws on the principal features that distinguishes each of the eastern samples from *X. muelleri* west.

The frontoparietal may occasionally be narrower anterolaterally than at the level of association with occipitopetrosals, lacking pronounced anterolateral corners (Illustration 6-6). Parasagittal crests are narrower, the dorsal skull table measuring approximately one third of the width of the element. The pineal foramen is more caudally positioned, occurring approximately half way along the length of the element. Retractor bulbi muscle scars (rtbs) are generally narrower, but widths are highly variable and ranges overlap (Illustration 6-7). Optic foramina are relatively large and visible in ventral view. The azygous vomer differs in generally being edentate and having concave anterior margin and deeply convex posterior margin, lateral margins being sub-parallel. The rostral nasal extension is almost always bulbous, having a prominent medial scar. Anterior margins of lateral nasal rami are sub-perpendicularly oriented with respect to the zonal plane in most specimens, occasionally resembling the perpendicular state seen in *X. muelleri* (west). Although absent in most, bilateral foramina occasionally occur in the lateral nasal extensions of some specimens. Expansion of clavicles towards their medial margin is greater than in western samples. With the exception of the ultimate pre-sacral vertebra, each bears paired neural spines (Illustration 6-8). Sacral diapophyses range from being symmetrical around the transverse plane (cf. *X. muelleri* [west]) to being highly expanded in a posterior direction. See also Illustration 6-9.

Evidence of hybridization with *X. l. laevis* in eastern parts of the *X. muelleri* distribution range

A striking similarity was evident between the characters that distinguish *X. muelleri* east from west and those that distinguish *X. muelleri* from *X. l. laevis*. The direction of variability of these characters in *X. muelleri* was assessed with respect to the state observed in *X. l. laevis* (see chapters 3 and 7). Results from this comparative study found many of these characters in *X. muelleri* east to approach the state expressed in *X. l. laevis*, suggesting the possibility of hybridization between these taxa in eastern parts of the *X. muelleri* range. Characters indicating introgression between *X. laevis* and *X. muelleri* (east) include position of parasagittal crests, which are narrower in *X. muelleri* east than west, approaching the state seen in *X. l. laevis*, in which taxon, crests form a narrow, parallel-sided dorsal skull table. The distance between retractor bulbi muscle scars (rtbs) in *X. muelleri* east approaches the state seen in *X. l. laevis*, as does vomer shape, edentate state of the vomer (*X. muelleri* being the only taxon documented as having a dentate vomer) and maximum vomer length. The shape

of sacral diapophyses in *X. muelleri* east resembles the state found in *X. l. laevis* more closely than that of *X. muelleri* west. The degree of medial clavicle expansion, total number of teeth and anterior sphenethmoid width in *X. muelleri* east, as compared with *X. muelleri* west, is consistent with introgression with *X. l. laevis* in eastern samples. Finally, the maxillary, which in *X. muelleri* west is typically square-ended, is tapered in the Skukuza sample, resembling the state seen in *X. l. laevis*.

A small number of characters that vary considerably between *X. muelleri* east and west do not directly support the theory of introgression with *X. laevis* in the eastern end of the *X. muelleri* distribution range, but neither do they preclude this theory. These include position of the pineal foramen, which in *X. l. laevis* is intermediate between eastern and western *X. muelleri* forms. Distance between otic plates varies in a cline between eastern and western *X. muelleri*, the distance between scars in *X. l. laevis* being significantly smaller than that of any of the *X. muelleri* samples. The angle separating coracoid and zonal plane varies along a cline, increasing from west to east with the exception of the Skukuza sample, which approaches the state seen in *X. l. laevis*, in which this angle is smaller than in western samples.

Principal characters that distinguish *X. muelleri* from other *Xenopus* taxa examined for the present study

Parasagittal crests are wider than in all other taxa except for *X. fraseri*. The pineal foramen in *X. muelleri* (east) is more caudally positioned than in any other taxon. The gap separating retractor bulbi muscle scars is higher than that in *X. laevis*, but equal to or narrower than that of *X. fraseri*. Though only universal in western forms, the dentate vomer distinguishes *X. muelleri* from all other taxa. The vomer shape, though different in eastern and western forms, differs from all other taxa. Orientation of anterior margins of occipitopetrosals is oblique in *X. muelleri* whilst generally being perpendicular to the zonal axis in other taxa. Otic plates are symmetrically bifid in *X. muelleri* whilst the caudal ala of the pair is larger than the rostrally directed ala in other taxa. Ventral occipitopetrosal crests are obliquely oriented in *X. muelleri*, whilst being perpendicular to the zonal plane in other taxa. A short, discrete process at the base of tympanosquamosal overlaps occipitopetrosals, not seen in any other taxon. The maxillary arch is longer than that of any other taxon, and is square-ended in contrast to the distally tapered maxillaries of other taxa. Septomaxillaries are relatively long, and are angled more obtusely with respect to the nasal than in other taxa. Paired neural spines occur either on the atlas alone or on all pre-sacral vertebrae in *X. muelleri*, whilst only otherwise being paired on the atlas of *X. laevis* (occasionally) and in *X. borealis*. Combined suprascapula and cleithrum are unusually short and do not overlap mid-dorsally as they do in *X. laevis* and some *X. fraseri* specimens, and the clavicle varies little in width along its length, in contrast to the medially expanded state seen in *X. laevis*, and to a lesser degree in all other taxa. Finally, the relative proportions of

proximal and distal prehallical elements is significantly different to that seen in any other taxon, the distal element measuring almost three times that of the proximal element.

DISCUSSION

The observed sexual dimorphism in snout-vent length and intra-specific difference in the magnitude of this variation is generally consistent with records from the literature (Kobel *et al.*, 1996), though Skukuza specimens were unusually small. Although the method of analysis precluded the possibility of analyzing sexes separately, males and females were ultimately identifiable in each scatter graph (Figure 6-3, Figure 6-5) and in the dendrogram (Figure 6-6) and were not found to vary significantly within the context of phenotypic variation between geographical groups.

Although there is a risk attached in drawing cross-taxon comparisons, characters identified as being the most discriminatory between eastern and western *X. muelleri* were assessed with respect to the form and direction of variation with age of these characters in *X. l. laevis* (see chapter 3). Only two were shown to vary significantly in this respect for *X. l. laevis*, the number of premaxillary teeth increasing with age and the angle between coracoid and zonal plane decreasing. The direction of variability in these two characters in *X. muelleri* is contradictory in terms of implying a difference in age between samples: data for the former indicate that specimens from the *X. muelleri* east sample are older than those from western localities, whilst data for the latter indicate the opposite. Whilst not proving conclusively that the differences identified between *X. muelleri* east and west are independent of the effects of age, the direction of variation in these characters in *X. muelleri* is not consistent with them varying with age in the same direction as has been shown for *X. l. laevis*. Although the form of age-related variation is unknown for *X. muelleri*, all specimens used in the present study were sexually mature, and there was no osteological evidence to indicate a significant difference in developmental stage between samples. No evidence presents itself to indicate that the observed variation does not represent true systematic distance between geographical groups, rather than representing age-related variation.

Results showed a clear distinction not only between *X. muelleri* east (Ndumo, Skukuza, Uvira) and west (Garamba, and Sir, Gueme, Namoundjoga), but also between each of their constituent geographical groups. Variation between groups, as shown by scatter graphs, was not linear however, and as such did not indicate a geographical cline between east and west. Indeed, even variation among samples from Ndumo, Skukuza and Uvira was not linear. Nevertheless, the general direction of variation among these samples was perpendicular to the main direction of variation separating the western sample from Garamba specimens, indicating an affinity within the former three samples that distinguished them from the latter two. The geographical margin separating these two forms appears to lie between Garamba National Park and Lake Tanganyika. Sample sizes in the

present study were small however, and should be increased if conclusions are to be drawn from results regarding biogeography and distribution. Furthermore, the number of localities in the current study is low and should be increased to include samples from Tanzania and other parts of the range in order to gain a fuller appreciation of the extent and form of variation across the entire range.

Although it might be expected that a degree of geographical variation would affect these samples, the extent of variation indicated as separating groups is not proportional to the distances separating localities. The distance between Skukuza and Ndumo is little over 200km, yet the degree of phenetic similarity between these samples is greater than that which separates the Skukuza sample from that of Uvira, thousands of kilometers distant. Whilst it is possible that inter-species hybridization with sympatric taxa may have caused this discrepancy, founder effects offer an alternative explanation. Either would account for non-linearity of variation among samples, but the former provides a more likely explanation for outliers identified by multivariate analysis.

Records of sympatry in or near these sites would provide information pertaining to the probability of hybridization having occurred recently. Sympatry has indeed been documented in Ndumo, where *X. muelleri* and *X. l. laevis* share a common habitat (Tinsley, pers. com.). Although hybridization is not documented as having occurred here, the sample comprised a number of specimens of intermediate morphology. Care was taken in the present study to include only pure *X. muelleri* morphotypes. However, results obtained from analysis of material collected from Skukuza, carried out subsequent to the current study (see chapter 7), have shown evidence of introgression with *X. l. laevis* at this site, and have even shown pure *X. muelleri* morphotypes to express *X. l. laevis* genes, giving reason for concern regarding samples used both from this site and others in the current study. Samples from Uvira were also mixed, though this record is ambiguous since samples from adjacent sites may have been pooled during collecting. Records of sympatry between *X. muelleri* and three other species (see intro.) are documented for Garamba national park. The sample from Sir, western Africa, was mixed in approximately equal proportions with *X. l. sudanensis*, and although the situation for the sample from Gueme is unknown, this locality is also on the border between *X. l. sudanensis* and *X. muelleri* ranges, and as such, mixing is likely to have occurred in the recent past if it is not occurring currently. The situation is unknown for Nyamoundjoga. However, this site is beyond the range of *X. laevis*.

The relative values of characters identified as varying most between samples in the current study were compared with corresponding values for *X. l. laevis*, the taxon most commonly documented in sites of sympatry in southern African localities (using the same sample as that used in chapter 7). Variation was generally consistent with the theory of there having been introgression of *X. l. laevis* genes into the eastern *X. muelleri* population, especially evident in Ndumo and Skukuza samples.

Although there is no published account of widespread hybridization between these two taxa, a single, sterile F1 hybrid was recently found in southeastern Africa (Fischer, Koch and Elepfandt, 2000) and a number of morphologically intermediate specimens have been identified in mixed species samples collected from Uvira and Ndumo (pers. obs.; Tinsley pers. com.). The distribution range of *X. muelleri* is amongst the widest of all *Xenopus* taxa, and borders that of *X. laevis* along most of its length, encountering *X. l. laevis* at the southern end of its range, *X. l. victorianus* in the Tanganyika area in the vicinity of Uvira and *X. l. sudanensis* in Cameroon. Whilst this distribution pattern provides the potential for hybridization with *X. laevis* along almost the entire length of its distribution arc, *X. laevis* is not recorded from northeastern (Tanzania) or western parts (Burkina Faso and Nyamoundjogo) of the *X. muelleri* range. Acquiring material from Tanzania would provide the potential to study further material for which there is low risk of contamination by *X. laevis* for comparison with samples used in the current study.

Little distinguishes the habitat preferences of *X. muelleri* and *X. laevis,* an observation that is consistent with their ranges overlapping broadly in the area of southeastern Africa that forms the focus of the following chapter. They share a generalist lifestyle that is understood to have been exacerbated by a history of considerable habitat disturbance (Fjeldsa, 1997). Hybridization may perhaps be an adaptation to this disturbance, providing the population with an increased degree of genetic plasticity that may enable tolerance of a greater range of environmental conditions (Fjeldsa and Lovett, 1997). Regardless of the forces behind this remarkable adaptation, the phenomenon defies our current understanding of the species concept, and makes *Xenopus* a remarkable group for the study of evolutionary process. Results from the following chapter are of significant interest in this respect and provide valuable insight for interpretation of results from the present study.

Regardless of the impact of hybridization on *X. muelleri*, the species will also have undergone repeated episodes of extreme habitat disturbance throughout the Pleistocene (Fjeldsa, 1997). The distribution range of *X. muelleri* will have expanded during glacial maxima, the most recent of which has been dated at 18,000yr BP (Hamilton, 1982; Tinsley *et al.*, 1996; see thesis introduction). The effects of vicariance on genetic diversification within the species will have been significant, having greatest impact on small groups owing to the effects of genetic bottlenecking (Hedberg, 1969). Whilst it is probable that founder effects have been important in the recent history of *X. muelleri* east and west, variation among eastern samples appears to have been exacerbated by inter-specific hybridization. Variation between eastern and western populations is considerably more pronounced than variation within each of these groups however, and as such is interpreted as being the result of more profound and prolonged separation. It is not clear however, where division of the *X. muelleri* distribution range occurred during the Pleistocene, since most research carried out to determine historical patterns of habitat change have focused on forest taxa (Wasser and Lovett, 1993). What little is known about changes in the distribution pattern of savanna across Africa is drawn from

inference relating to changes in the distribution of forest. Nevertheless, it is clear that the western rift has long posed a formidable barrier to migration, and continues to do so today (Hamilton, 1982; Lovett, 1993; Fjeldsa, 1994). Lowland tracts identified as providing potential migration routes for forest taxa between eastern and western areas of the Western Rift during interglacial periods may have provided routes for savanna species during glacials. These are located in northern Kenya and through the Tanganyika Rift, along valleys towards Rukwa rifts (Hamilton, 1976; Wasser and Lovett, 1993).

CONCLUSION

A considerable degree of osteological variation distinguishes each of the samples included in the present study. The most profound distinction is that which separates eastern samples (Ndumo, Skukuza, Uvira) from those in the west (Garamba, Sir, Nyamoundjoga, Gueme), although the degree of variation exhibited within each of these groups is also considerable. Whilst it is acknowledged that more samples are required before an evaluation can be made regarding the overall pattern of variation within *X. muelleri*, evidence from the current study indicates that the barrier preventing interaction between eastern and western populations has been greater than that separating their constituent groups, and appears to lie between Garamba and Lake Tanganyika. This barrier is likely to have taken the form of intermittent geographical isolation caused by habitat fragmentation, accentuated during interglacial periods throughout the Pleistocene. Evidence of osteology indicates that in addition to vicariance, hybridization also appears to have affected the eastern sample, though further fieldwork in one of the documented areas of sympatry in southeastern Africa is needed to corroborate this interpretation.

BIBLIOGRAPHY

Burki, E. and Fischberg, M. (1985). Evolution of globin expression in the genus *Xenopus* (Anura: Pipidae). *Molecular Biology and Evolution* **2**:270-277.

Corruccini, R.S. (1987). Univariate versus multivariate morphometric variation: an alternative viewpoint. *Systematic Zoology* **36(4)**:396-397.

Fischer, W.J., Kock, W.A. and Elepfant, A. (2000). Sympatry and hybridization between the clawed frogs *Xenopus laevis laevis* and *Xenopus muelleri* (Pipidae). *Journal of Zoology, London* **252**:99-107.

Fjeldsa, J. (1994). Geographical patterns for relict and young species of birds in Africa and South America and implications for conservation priorities. *Biodiversity and Conservation* **3**:207-226.

Fjeldsa, J. and Lovett, J.C. (1997). Biodiversity and environmental stability. *Biodiversity and Conservation* **6**:315-323.

Fjeldsa, J. (1997). Geographical patterns of old and young species in African forest biota: the significance of specific montane areas as evolutionary centres. *Biodiversity and Conservation* **6**:325-346.

Graf, J.-D. and Fischberg, M. (1986). Albumin evolution in polyploid species of the genus *Xenopus*. *Biochemical Genetics* **24**: 821-837.

Hamilton, A.C. ed. 1982. Environmental History of Africa: A Study of the Quaternary. London: Academic Press.

Hamilton, A. (1976). The significance of patterns of distribution shown by forest plants and animals in tropical Africa for the reconstruction of upper Pleistocene Palaeoenvironments: A Review. *Palaeoecology of Africa* **9**:63-97.

Hedberg, O. (1969). Evolution and speciation in a tropical high mountain flora. *Biological Journal of the Linnean Society* **1**:135-148.

Inger, R.F. (1968). Amphibia- General description of the Garamba National Park, Parc national de Garamba. Mission H. De Saeger. fascicule 52.

Kobel, H.R., Loumont, C. and Tinsley, R.C. (1996). "The extant species." Pp. 9-33 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Kobel, H.R., Barandun, B. and Thiébaud, ChH. (1998). Mitochondrial rDNA phylogeny in *Xenopus*. *Herpetological Journal* **8 (1)**:13-18.

Loumont, C. (1984). Current distribution of the genus *Xenopus* in Africa and future prospects. *Revue Suisse Zoologie* **91 (3)**:725-746.

Loumont, C. (1986). *Xenopus pygmaeus*, a new diploid pipid frog from rain forest of equatorial Africa. *Revue Suisse Zoologie* **93**:755-764.

Lovett, J.C. 1993. "Climatic history and forest distribution in eastern Africa." Pp. 23-29 in Biogeography and Ecology of the Rainforests of Eastern Africa, edited by Wasser, S.K. and Lovett, J.C. cambridge: Cambridge University Press.

Mann, W., Risley, M.S., Eckhardt, R.A. and Kasinsky, H.E. (1982) Characterization of sperm/spermatid basic chromosomal proteins in the genus *Xenopus* (Anura: Pipidae). *Journal of Experimental. Zoology* **200**:173-186.

Muller, W.P. (1966). Diplotene chromosomes of *Xenopus* hybrid oocytes. *Chromosoma* **59**:273-282.

Peters, W. (1844). Inachricht von einigen Fischen u. Amphibien aus Angola u. Mozambique. *Monatsberichte der preussischen akademie der wischenschaften au Berlin* **1844**:32-37.

Poynton, J.C. (1962). Zoogeography of Eastern Africa: An outline based on anuran distribution. *Nature* **194**:1217-1219.

Poynton, J.C. (1964). The Amphibia of South Africa: A faunal study. *Annals of Natal Museum* **17**:1-334.

Schiotz, A. (1963). The amphibians of Nigeria. *Videnskabeligue Meddelelser fra Dansk Naturhistorisck Forening* **125**:1-92.

Tinsley, R. C. (1981a). The evidence from parasite relationships for the evolutionary status of *Xenopus* (Anura: Pipidae). *Monitore Zoologico Italiano***19**:367-385.

Tinsley, R. C. (1981b). Interactions between *Xenopus* species (Anura: Pipidae). *Monitore Zoologico Italiano* **9**:133-150.

Tinsley, R.C. 1996. "Parasites of *Xenopus*." Pp. 233-251 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Tinsley, R.C. and Jackson, J.A. (1998). Correlation of parasite speciation and specificity with host evolutionary relationships. *International Journal for Parasitology* **28**:1573-1582.

Tinsley, R.C., Loumont, C. and Kobel, H.R. 1996. "Geographical distribution and ecology". Pp. 35-59 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Wasser, S.K. and Lovett, J.C. 1993. "The Geological Evolution of East Africa." Pp. 30-47 in Biogeography and Ecology of the Rainforests of Eastern Africa. : Cambridge University Press.

Willig, M.R., Owen, R.D. and Colbert, R.L. (1986). Assessment of morphometric variation in natural populations: the inadequacy of the univariate approach. *Systematic Zoology* **35(2)**:195-203.

CHAPTER 7 : EVIDENCE OF NATURAL HYBRIDIZATION AND INTROGRESSION BETWEEN *XENOPUS LAEVIS LAEVIS* AND *XENOPUS MUELLERI* (EAST) IN SOUTHERN AFRICA

INTRODUCTION

The present chapter is concerned with the interaction between two savanna taxa, *Xenopus muelleri* and *X. laevis laevis* in an area of sympatry in southern Africa. Until relatively recently, *X. muelleri* and *X. l. laevis* were believed to be largely allopatric, their ranges separated by an abrupt transition zone (Poynton, 1964; Poynton and Broadley, 1985; Poynton and Boycott, 1996; Kobel, Kobel, Loumont and Tinsley, 1996; Tinsley, Loumont and Kobel, 1996). The distribution of *X. laevis* extends from the Cape in the south to Nigeria in the northwest and to Sudan and Kenya in the northeast. That of *X. muelleri* (east) extends throughout lowlands of east and southeast Africa, in biotypes characterized by elevated temperatures (see previous chapter; Kobel *et al.*, 1996; Tinsley *et al.*, 1996). An altitude differential generally separates these two species in areas where they occupy adjoining habitats. *Xenopus muelleri* occurs at altitudes up to 800m in a biotype characterized by short grassland, open bush-land and scrub forest, whilst *X. l. laevis* occupies higher altitude bush, tall grass and open parkland (Poynton and Boycott, 1996; WWF, Ecoregion map, 1998). The distribution of *X. l. laevis* appears to be restricted at the lower end of its range by *X. muelleri* (Passmore and Carruthers, 1995), tolerating much higher temperatures however in Namibia, an area beyond the range of *X. muelleri* (see Tinsley *et al.*, 1996). This precludes the theory that a thermal constraint restricts the distribution range of the former. Constraints are more likely a function of a range of environmental and biotic factors, including competitive interaction.

The abrupt transition zone previously reported by Poynton (1964) as separating the ranges of these taxa has since been found to contain extensive areas of sympatry (Poynton and Boycott, 1996). Although Poynton and Broadley (1985) reported having found specimens of intermediate morphology in this zone, none was found during subsequent fieldwork by Lambiris (1988). Only recently, in a paper documenting the capture of a wild-caught, F1 hybrid from Southeastern Africa, was there published documentation of natural hybridization between these two and of their co-occurrence in the same water-body (Fischer, Koch and Elefandt, 2000).

Reports of natural hybridization in *Xenopus* are rare, documented as having occurred only between closely related *X. gilli* and *X. l. laevis* in the Cape of South Africa (Rau, 1978; Kobel, Du Pasquier and Tinsley, 1981; Picker, 1985; Picker, Harrison and Wallace, 1996), between *X. borealis* and *X. l. victorianus* in Kenya, based on evidence of morphology alone (Yager, 1996) and between *X. l. laevis* and *X. muelleri* in Southeast Africa (Poynton and Broadley, 1985; Fischer *et al.*, 2000).

The ranges of Afromontane habitats in eastern South Africa are known to have undergone expansion, contraction and fragmentation throughout the Pleistocene (Cooke, 1962; Stuckenberg, 1969; Acocks, 1975; Van Zinderen-Bakker, 1978; Diamond and Hamilton, 1980; Lawes, 1990), although to what degree remains contentious. The ranges of neighbouring, lowland habitats would inevitably have undergone reciprocal changes. With these habitats representing parts of *X. l. laevis* and *X. muelleri* ranges respectively, it can be assumed that their distributions will have undergone corresponding shifts. As such they will have repeatedly come into contact throughout the Pleistocene, providing a series of opportunities for inter-species interaction at species boundaries. Evidence of hybridization between these species in the current zone of parapatry is suggestive of ancient, if intermittent, hybridization whenever their ranges have come into contact.

In the only comprehensive account of natural hybridization between *X. muelleri* and *X. l. laevis* (see Fischer *et al.*, 2000), the single F1-hybrid was found to be infertile, perhaps not surprising given the high degree of taxonomic differentiation known to exist between *X. muelleri* and *X. l. laevis* (see Graf, 1996; Kobel *et al.*, 1998) and low rate of bivalent formation at meiosis demonstrated in hybridization experiments by Muller (1966). Despite each of these approaches indicating a low probability of hybrids being fertile, none precludes the possibility altogether. Evidence from the field and from breeding experiments is needed to resolve the matter of *X. muelleri* x *X. l. laevis* hybrid fertility.

Fieldwork

In December 1999, R. Tinsley, J. Jackson, L. DuPreez and I carried out fieldwork along a transect that intersects the contact zone between the ranges of these two species. The transect cuts perpendicularly through coastal and inland contours, incorporating the convergence of east African lowland and Afromontane ecotypes, along the Drakensberg escarpment (Poynton and Boycott, 1996). The area is one of significant biogeographical interest and is characterized by a transitional assemblage of amphibian fauna (Poynton and Boycott, 1996). Whilst the Swaziland transect extends from the Mozambique border in the northeast to the highveld border with Eastern Transvaal in the northwest, sampling was also carried out at a range of sites in the Kruger National Park and areas south of the park boundary.

The morphology of *X. l. laevis* and *X. muelleri* is very similar, and despite there being a considerable degree of intra-species variation, a small number of external characters enable unambiguous identification of each. Where there is evidence of hybridization however, taxonomic distinction using morphology alone becomes problematic. A variety of alternative methods can be applied to the determination of taxonomic status in hybrid zones. Whilst external morphology is commonly used and offers the most immediate means of categorization (Moore and Buchanan, 1985; Gollmann, Roth and Hodl, 1988; Sullivan, 1995; Evans, Morales, Picker, Melnick and Kelley, 1998;

Klingenberg *et al.*, 2000), the method relies on there being significant and consistent inter-species variation between the populations concerned. Comparative osteology has previously been applied as a method for studying a hybrid zone between two *Geocrinia* species (Anura), but failed to distinguish conclusively between parent taxa (Gollmann, 1991). Biochemical, cytological and mate-call traits each provide additional means of taxonomic determination (Fischer *et al.*, 2000) whilst the prevalence of osteological deformities provides information pertaining to genetic fitness, which is said to be compromised in hybrids (Littlejohn, Watson and Loftus-Hills, 1971; Gollmann, 1991). Breeding experiments and prevalence of parasite infection (Le Brun, Renaud, Berrebi and Lambert, 1992) could further enhance our understanding of hybrid-zone dynamics.

AIM

Samples collected during fieldwork were examined with a view to identifying hybrids and distinguishing them from parent taxa. A range of techniques will be employed to determine taxonomic status of specimens and to establish whether there is evidence of hybrid fertility. Methods will include comparative morphology, osteology, biochemistry, flow-cytometry, mate-call analysis and breeding experiments.

Locality data will be assessed to establish whether the hybrid zone occurs only along a narrow geographic zone that clearly defines the margins of parent species' distribution ranges, or indeed whether the effects of hybridization are more widespread across a more diffuse zone of contact. Correlating altitude with distribution data will reveal to what degree altitude determines the distribution of hybrids. Any potential threat to the genetic integrity of parent species will also be assessed, with reference to evidence of past periods of contact between species ranges.

MATERIALS AND METHODS

Fieldwork

A total of 519 *Xenopus* was collected using modified bucket traps, by net sweeping ponds and by hand during fieldwork carried out in December 1999 in South Africa and Swaziland. Grid references and altitude data were collected where possible, using a GPS. Where altitude was not recorded by GPS, it was estimated from a 1:1,000,000 scale air navigation map of the area; where this was the case, altitudes have been marked 'est.'. Although water temperature was recorded at most sites, data were not directly comparable between sites, since recordings were made at different times of day. Some specimens were fixed in the field by immersion in the anesthetic, MS222 prior to fixation in 10% formalin, others were returned live to the laboratory in Bristol.

Locality Details

Italicized locality details refer to sites where no specimens were captured.

Kruger National Park and South Africa:

1. Skukuza camp. 24°59'10"S, 31°35'08"E. Alt. 346m.
2. Pretoriuskop, 27°C. 25°10'06"S, 31°15'58"E. Alt. 533m.
3. Fayi Loop, 25.8°C. 25°12'05"S, 31°15'39"E. Alt. 643m.
4. Nr. Fayi Loop, 30.3°C. 25°14'02"S, 31°15'45"E. Alt. 554m.
5. Tributary of Nsikani, 30.7°C. 25°14'50"S, 31°15'24"E. Alt. 616m.
6. Tributary of Nsikani, 25.5°C. 25°16'04"S, 31°15'25"E. Alt. 465m.
7. Tributary of Nsikani, 26.5°C. 25°16'35"S, 31°15'58"E. Alt. 416m.
8. *Heading north to Pretoriuskiop, 25.1°C. 25°12'07"S, 31°14'09"E. Alt. 562m.*
9. *Dam near Numbi gate, 27.8°C. 25°09'02"S, 31°11'41"E. Alt. 650m.*
10. *Roadside pool, 23°C. 24°58'22"S, 31°23'09"E. Alt. 401m.*
11. 25km from Skukuza, 29.6°C. 24°58'19"S, 31°23'24"E. Alt. 407m.
12. *N4, near Malalane - farm dam, 27°C. 25°29'37"S, 31°31'19"E.*
13. *Farm dam- side road off N4, 27.4°C. 25°29'19"S, 31°32'37"E. Alt. 551m.*
14. *Sugar factory, 29.3°C. 25°28'01"S, 31°33'57"E. Alt. 324m.*
15. *Mango farm (Alphick) off N4. 25°31'00"S, 31°26'13"E. Alt. 297m.*
16. Mango farm (Alphick) off N4. 25°30'44"S, 31°26'27"E. Alt. 434m.
17. *N4, near Nelspruit. 25°30'00"S, °31'10"30E.*
18. Mabalel hotel swimming pool. 25°29'28"S, 31°06'25"E. est. 609m.
19. *Crocodile Park. 25°27'30"S, 30°57'59"E.*
20. Dam in Fish farm nr. Nelspruit. (Chris Fouche). 25°26'53"S, 31°02'51"E. est. 609m.
21. Crocodile Park new dam. 25°27'30"S, 30°57'59"E. est. 650m.

Swaziland

22. 10km south of Piggs Peak. 26°02'09"S, 31°11'45"E. Alt. 1040m.
23. 30km south east of Piggs Peak. 26°03'29"S, 31°26'30"E. Alt. 700m.
24. *Roadside waterhole. 26°02'08"S, 31°38'13"E.*
25. Lowveld waterhole. 26°02'34"S, 31°38'12"E. Alt. 360m.
26. Nkambeni swimming holes. 26°03'05"S, 31°44'22"E. Alt. 335m.
27. 2 quarry pools. 25°59'25"S, 31°44'22"E. Alt. 320m.
28. Mbuluzi Game Reserve. 26°08'14"S, 32°00'31"E. Alt. 140m.
29. *Pool in land adjacent to Mbuluzi. 26°08'34"S, 32°00'04"E. Alt. 150m.*

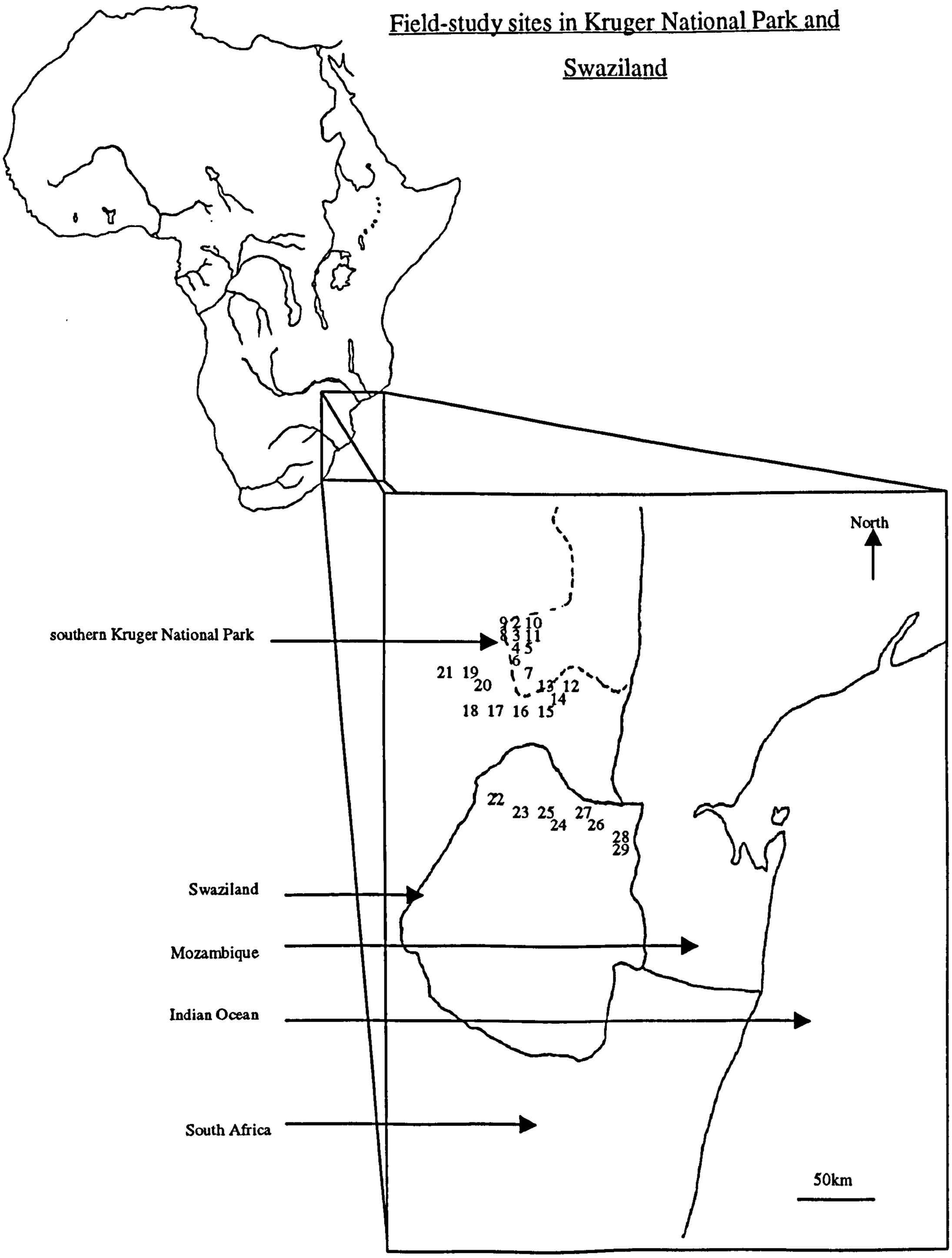


Figure 7-1

Map of southern Africa showing field-site localities.

External morphology

Introduction

A number of characters demonstrate distinct inter-species variation, and can be used for unambiguous distinction between parent taxa. These and others have been assessed in the field sample in an attempt to characterize specimens of intermediate taxonomic status.

Hybrid index (HI)

Following Sullivan and Lamb (1988), the degree of expression of nine characters was determined, five of which were used to generate a hybrid index (Table 7-2): sub-ocular tentacle length (*sot*), metatarsal tubercle (*mtt*), metacarpal length (*mcl*), length of mandible (*lm*), and eye position (*ep*). Inter-species variation in the remaining four characters was too great for them to be of practical use in the distinction between taxa (dorsal and ventral colours, eye colour and prominence). Of the former five characters, a numerical score was assigned to each state, the greatest corresponding to the state found in *X. muelleri* (long *sot, mtt,mcl, lm* and laterally positioned eyes). Pure *X. muelleri* yielded a score of 16 (1x4, 4x3) and pure *X. laevis* 5 (5x1). All references hereafter to 'pure species' refer to HI scores of either 5 or 16. All field specimens were scored twice, non-consecutively. Where these scores differed, specimens were re-assessed for confirmation.

Morphometrics

Snout-vent length was measured for all field specimens to the nearest 1mm, using Mitutoyo digimatic calipers. A further eleven external characters were chosen as representing the greatest inter-species variation between *X. muelleri* and *X. laevis*, and were measured using a binocular dissecting microscope fitted with an ocular micrometer. These were eye diameter (*ed*), inter-eye distance (*ied*), eye-to-eye inclusive (*etei*- distance between outer margin of lateral line plaques on each eye), width of head at anterior (*wha*) and posterior (*whp*) eye margins, length (*lj*) and width (*wj*) of jaw, carpal II (*mc*), radio-ulna (*ru*), sub-ocular tentacle (*sot*) and metatarsal tubercle (*mtt*) lengths. Each variable was standardized against snout-vent length and $\log_e$-transformed prior to conducting statistical analysis.

Methods

Statistical analysis

Data were standardized against s-v length and $\log_e$-transformed to render variation more linear and to eliminate differences in scale between variables (Klingenberg *et al.*, 2000). Principal component analysis (PCA) was used to investigate morphometric variability within the sample, independent of taxonomic assignment, and to resolve the relative contributions of size and shape in overall variation (Scriven and Bachau, 1992). PC1 mainly represented size-related variation, PC2 and 3, shape variation.

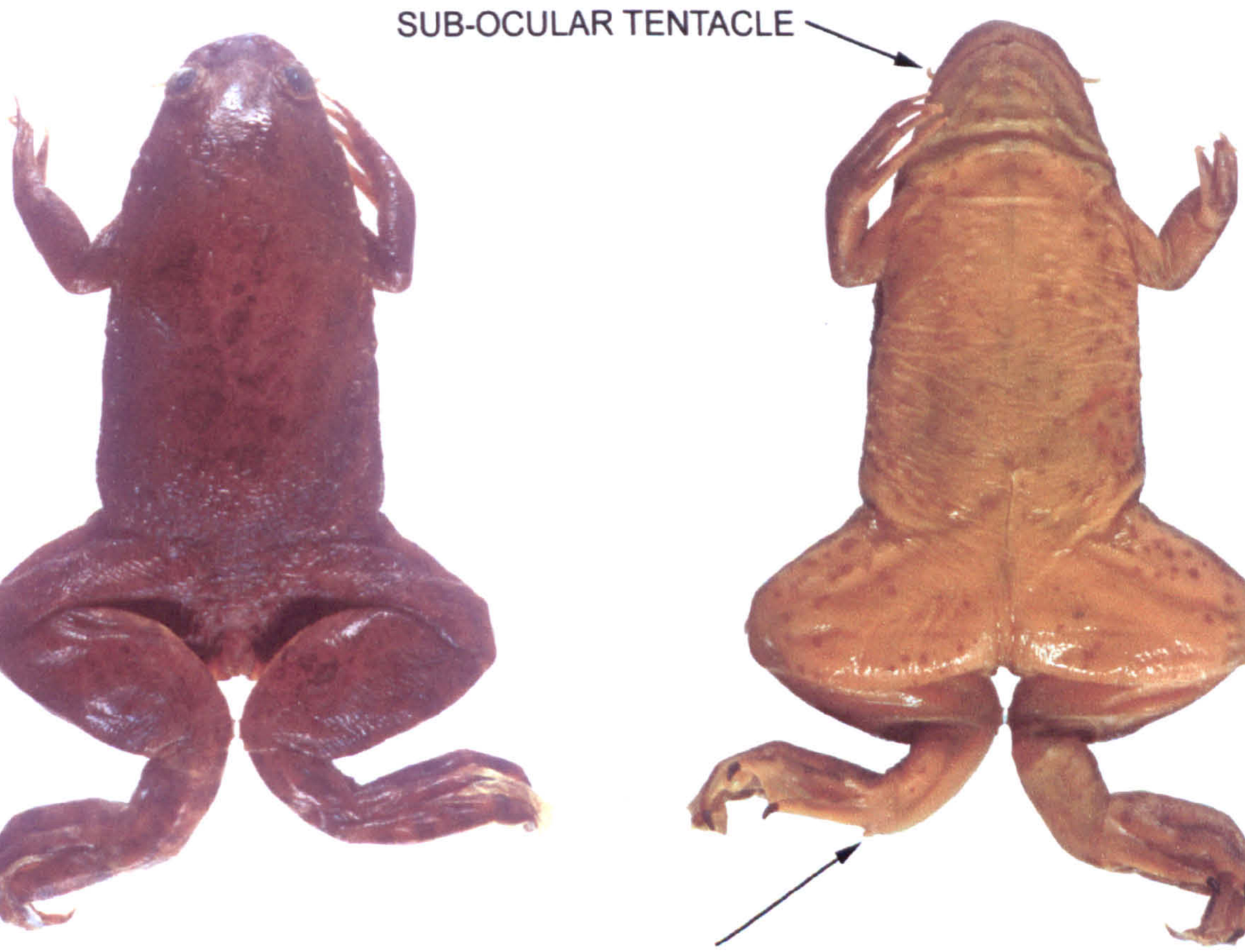


Figure 7-2

Photograph of *X. muelleri* (dorsal and ventral view) demonstrating morphological characteristics of the taxon.

Discriminant function analysis (DFA) (procedure: stepwise selection) maximized between sample variance, whilst minimizing within sample variance and was used to discriminate between pure species samples. The position of intermediates (specimens with intermediate III score) relative to those of pure species samples was also determined and a minimum set of variables identified for this level of distinction. Classification rates were calculated to provide an indication of the degree of morphological differentiation separating taxa (See methods section, chapter 3 for full account of statistical methods).

Results

A significant difference in snout-vent (s-v) length separated each group (F=41.3, d.f.=2, 433, p=0.000), and showed non-overlapping 95% confidence intervals. Intermediates showed a mean s-v length intermediate between that of pure taxa, *X. l. laevis* being the largest (Table 7-1).

	Mean s-v (male, female)	Min. (mm) (male, female)	Max. (mm) (male, female)
X. laevis (n=316)	64 (60, 67)	40 (40, 40)	99 (75, 99)
X. muelleri (n=76)	52 (48, 55)	41 (41, 42)	72 (59, 72)
Intermediate (n=44)	59 (54, 63)	38 (41, 38)	82 (65, 82)

Table 7-1

Mean (and range) s-v length of each sex for both taxa and intermediates.

Owing to an inter-sample size-bias, PCA was first carried out on external morphometric data to test whether the first component was dominated by a growth factor, or whether relative variation in shape exceeded that of overall size (Corti and Thorpe, 1989). All except for two characters had positive loadings of similar magnitude for the first principal component, which explained 61% of the variation and was interpreted as representing size-related variation; the two characters with negative loadings were sub-ocular tentacle and metatarsal tubercle, both larger in *X. muelleri*, the smaller taxon, than in *X. laevis*. Shape variation was explained by subsequent components, PC2 and 3, each also heavily weighted for the two aforementioned characters.

Second and third principal components (representing shape variation) were plotted against one another (Figure 7-3), and although species were broadly separated along the x-axis (PC2), distinction was imperfect, and the identification of intermediates not possible.

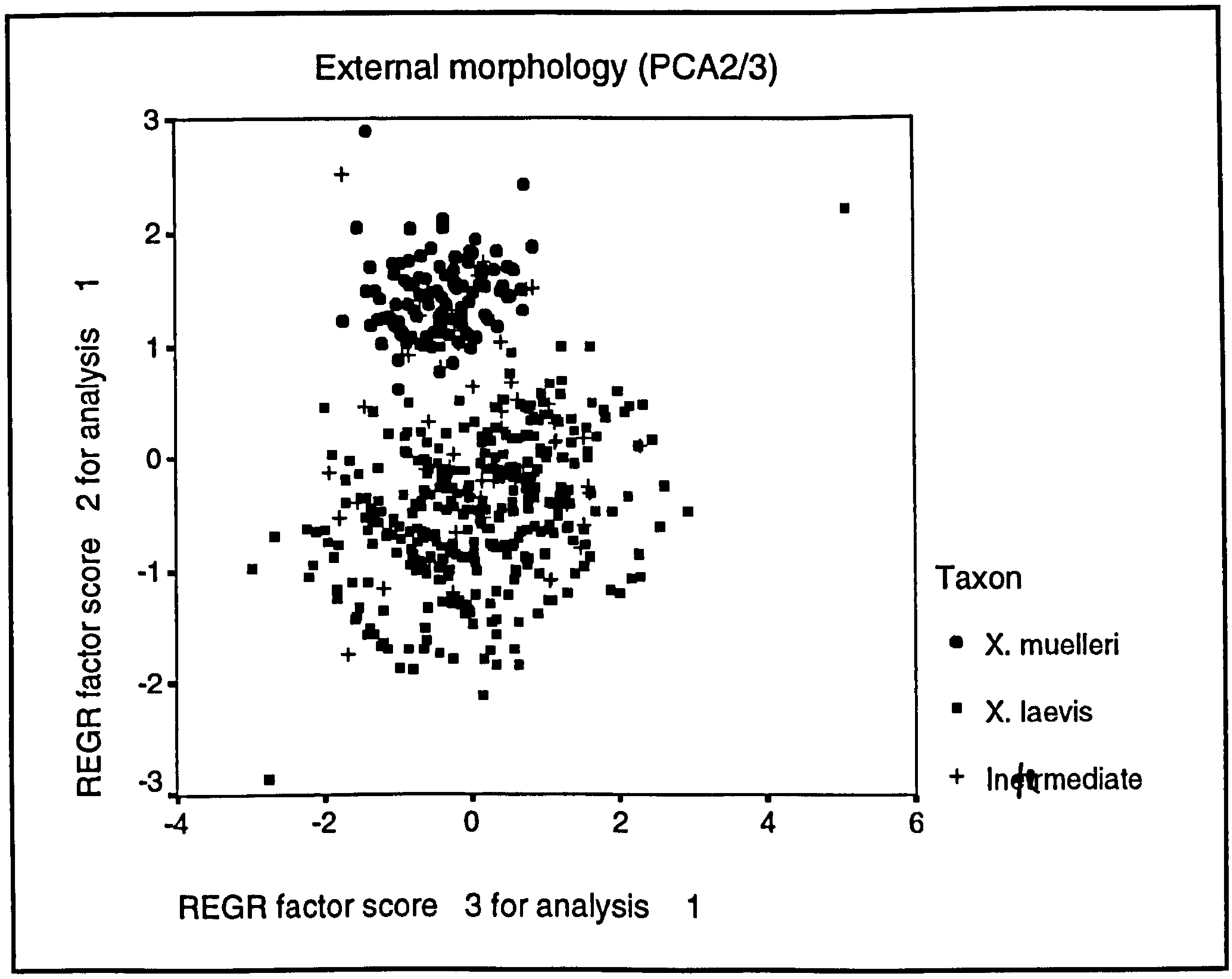


Figure 7-3

PCA 2 vs. 3 representing variation in shape between *X. muelleri* and *X. l. laevis* and intermediates based on external morphometric data. (REGR= PCA regression)

Stepwise DFA was carried out on the same data and yielded a single significant function (F=971, d.f.=1, 452, p=0.000). Neither the rate of correct classification nor the probability of group membership was altered by reducing the number of variables to the following, given in abbreviated form: *ed, ete, etei, wha, whp, sot, mtt* and snout to vent length (*s-v*), which together accounted for most of the observed variation in shape.

This function placed 88% of pure specimens within their correct group with over 99.9% confidence, but owing to the overlapping nature of component scores for each group, the identification of intermediates remained elusive. Specimens of intermediate HI status were each given a probability value for membership to either pure species group; all except for four of these were placed with a high degree of confidence (over 99.5%), within one of the pure species groups. These results were used with HI scores to confirm pure species status for some specimens whilst identifying potential intermediates for use in a subsequent, osteological study.

Figure 7-4 shows HI scores plotted against discriminant scores (DFA of PCA factors 2-5). Cases are identifiable by individual labeling, omitted here to preserve visual clarity.

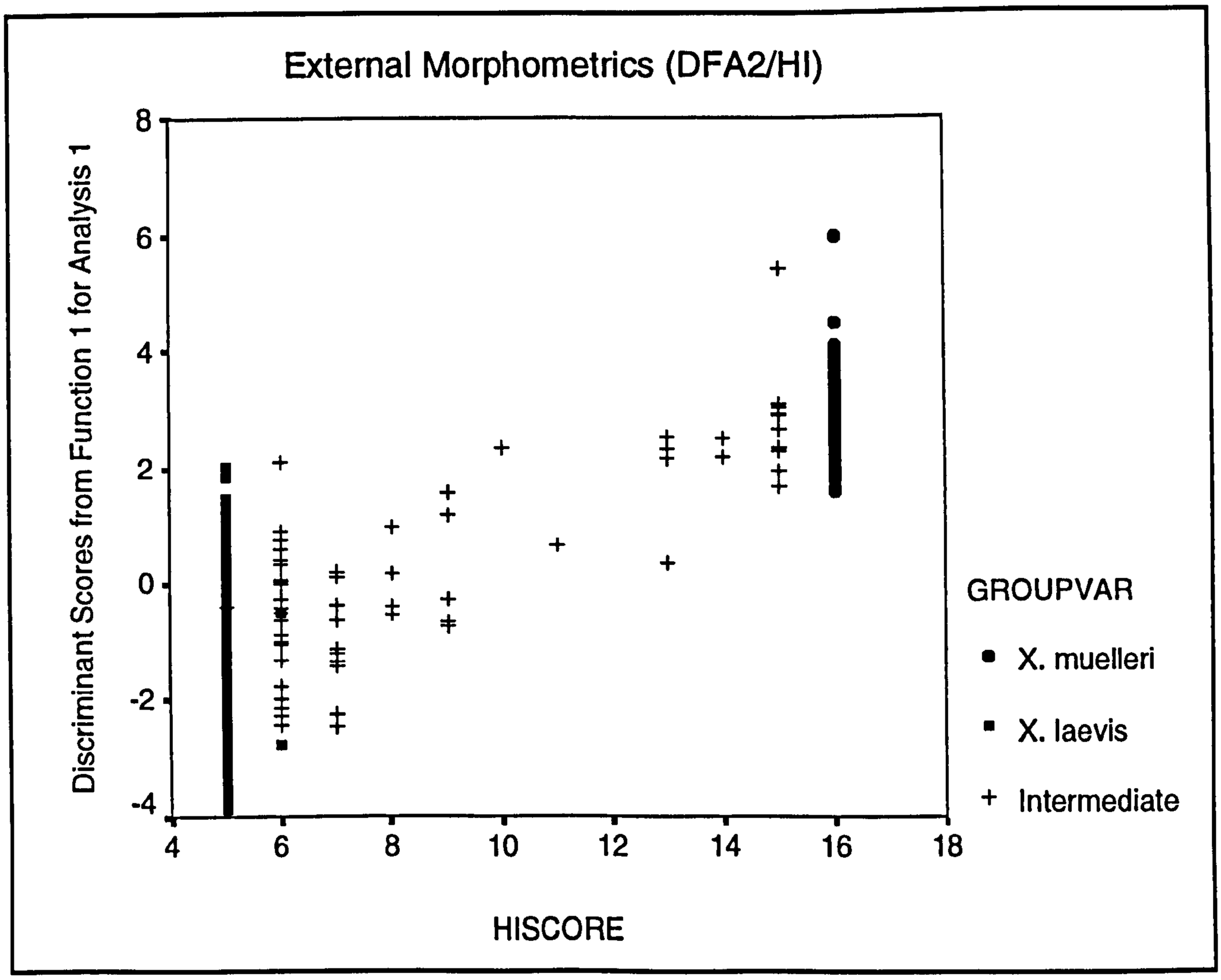


Figure 7-4

DFA (of PCA 2-5) vs.HI score- representing variation in shape between *X. muelleri* and *X. l. laevis* and intermediates based on external morphometric data.

Results from these analyses were consistent in identifying a core set of specimens whose external morphology was indicative of a pure taxonomic status. In contrast, none was unambiguously able to identify specimens with intermediate morphology. A small set of specimens, however, consistently fell in the boundary between these pure samples. Where these formed part of the fixed sample, they were subsequently used for osteological analysis. Where they formed part of the live sample, specimens were used in mate-call and comparative biochemical and flow-cytometry studies.

Distribution data

Introduction

Given the premise that where pure species ranges adjoin, the altitude threshold separating their respective distributions is said to lie at approximately 800m (Kobel *et al.*, 1996), results from morphometric analyses were used to map the distribution of pure and mixed species sites for comparison with sites that contained specimens of intermediate morphology, to determine the degree of correlation with altitude.

Results

Data, based on HI scores of external morphology, are presented in the form of a frequency table, listing how many specimens were collected at each site.

Site number	Altitude (m)	Taxonomic Category	*X. muelleri*	*X.l.laevis*	Ints.
1	346	Mixed and intermediate	6	33	50
2	533	*X. muelleri* and intermediate	46		6
3	643	Mixed	1	2	
4	554	*X. muelleri*	1		
5	616	Mixed	1	1	
6	465	Intermediate			1
7	416	*X. muelleri* and intermediate	3		2
11	407	Intermediate			1
16	434	Mixed and intermediate	3	2	1
18	609 (est.)	Intermediate			6
20	609 (est.)	Mixed	2	36	
21	650 (est.)	*X. l. laevis*		1	
22	1040	*X. l. laevis* and intermediate	115		7
23	700	Mixed and intermediate	2	112	20
25	360	*X. muelleri*	2		
26	335	Mixed and intermediate	38	3	4
27	320	Mixed and intermediate	6	2	1
28	140	*X. muelleri*	2		

Table 7-2

Altitude data for sites containing pure and mixed species, and sites containing specimens of intermediate morphology. (Int.= specimens of intermediate morphology)

Of a total of 18 sites, four are pure species sites. Two of these contain only a single specimen and the remainder, only two; little significance can therefore be attached to this. Only three sites contain both pure taxa without also containing specimens of intermediate morphology, two of these have sample sizes too small to be significant in this respect. Eleven sites contain specimens of intermediate morphology, albeit two of these are single specimen sites. Mixed-species sites ranged in altitude from 320m-700m, with evidence of hybridization extending to the highest site within our sampling range, 1040m. That the sample at the lower end of this range, at 140m, is a single species site may be at least partly attributable to it containing only two specimens. This pattern of predominantly mixed species sites demonstrates no strong relationship with altitude and will have provided ample opportunity for natural hybridization to have occurred across the study area.

Osteological morphometry

Introduction

Xenopus muelleri (east) and *X. l. laevis* are known to differ significantly in respect of their osteology (pers. obs.; see chapters 3 and 6). The maxillary arch is half the length of the skull in *X. muelleri* whilst measuring only a quarter to one-third the skull length in *X. l. laevis*. The distal prehallux element measures twice the length of the proximal element in *X. muelleri* whilst measuring approximately the same as the distal element in *X. l. laevis*, and the position of the pineal foramen in *X. muelleri* is almost half way along the frontoparietal whilst occurring within the anterior third in *X. l. laevis* (for further details, see individual osteological accounts in other chapters). Comparative osteology was therefore applied as a means of determining between parent species and specimens of intermediate taxonomic status.

Materials and methods

Specimens were selected for use according to HI scores and results from statistical analysis of external morphometric data. Specimens with HI scores of 5 or 16 and DFA (external morphometry) scores that indicated pure taxonomic status were selected from apparently pure species sites where possible (Skukuza for *X. muelleri* and Piggs Peak for *X. l. laevis*), whilst specimens demonstrating intermediate morphology (HI and DFA) were selected from a range of localities (Site numbers 1, 2, 6, 7, 23).

Twelve of each species (six of each sex) and 9 specimens of intermediate morphology (3 male specimens) were cleared and stained for examination (following methods outlined in chapter 3), their snout-vent lengths measured (Table 7-3). Owing to small sample size, it was not possible to conduct multivariate analyses separately for each sex, however characters determined to be of taxonomic importance were each retrospectively assessed for sexual dimorphism.

	Mean s-v (male, female)	Min. (mm) (male, female)	Max. (mm) (male, female)
X. laevis (n=12)	61 (58, 64)	56 (56, 60)	69 (61, 69)
X. muelleri (n=12)	52 (49, 56)	47 (47, 51)	61 (52, 61)
Intermediate (n=9)	57 (59, 56)	43 (59, 43)	65 (61, 65)

Table 7-3

Snout-vent length for each group, with data on sexual dimorphism.

Seventy-four characters were measured, at a magnification of x6.5, using a binocular dissecting microscope fitted with a calibrated eyepiece graticule. Characters are listed in Appendix 1, chapter 1. Statistical analysis followed methods outlined in chapter 2.

Results

Osteometric data were analyzed using PCA, carried out to determine the magnitude of the growth component in total osteological variation. PC1 explained 53% of the total variation and the majority of characters had positive loadings of similar magnitude. Inter-otic plate distance and the angle separating zonal-plane and coracoid, each had negative loadings and were significantly greater in *X. muelleri*, the smaller species, than in *X. l. laevis* (T=-5.2, d.f.=21, p=0.000). The first component was therefore interpreted as mainly representing size variation.

Remaining components (PCA 2-5) represented shape variation exclusively, and were also heavily weighted for the two aforementioned characters. Whilst PCA 2 and 3, plotted against one another, failed to distinguish clearly between pure species groups, DFA (on PCA 2-5), showed there to be a clear distinction. Furthermore, this method enabled the identification of a number of specimens displaying intermediate morphology. DFA1 (of PCA 2-5) was plotted against HI score to illustrate the high degree of congruence between results from the HI scoring method and DFA for osteology (Figure 7-5). Of those specimens originally designated by the HI scoring method as intermediate, most also showed an intermediate DFA coefficient (Y axis), and as such are interpreted as being of intermediate taxonomic status.

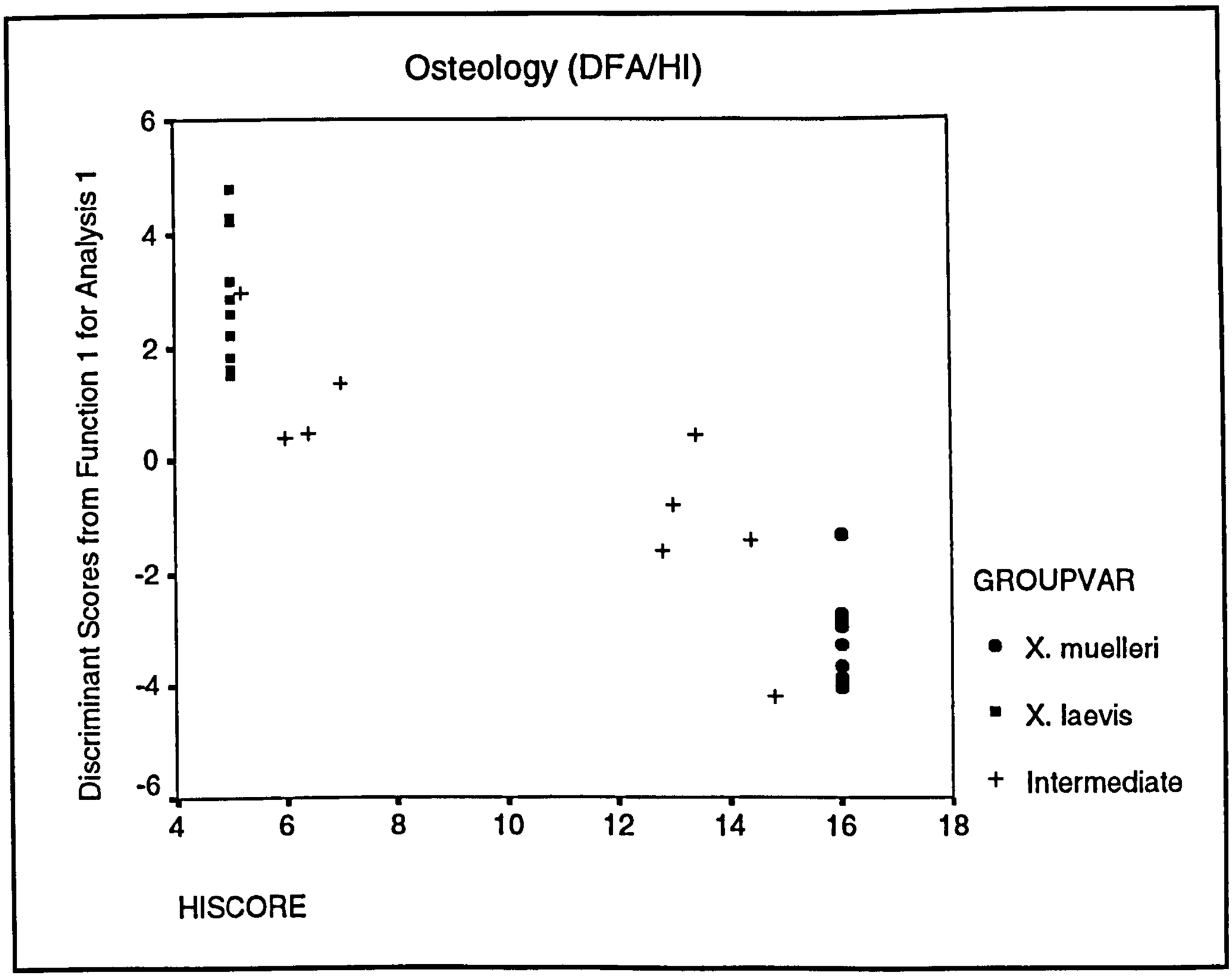


Figure 7-5

DFA osteology plotted against HI score

Raw data were re-examined using DFA (procedure: stepwise) to identify a minimum number of variables that could be used to distinguish between species and to enable a more convenient method for diagnosis in future studies. Variables with lowest Wilk's λ values were sequentially removed by this method. DFA continued to yield significant functions using only two of the original variables (d.f.=1,22; F=140; p=0.000), whilst still giving a 100% rate of correct classification. Furthermore, the probability levels for group membership of specimens rated as intermediate were not altered by sequential removal of these characters. The two characters remaining in the final analysis were inter-otic distance and length of the frontoparietal posterior to the pineal foramen (see illustrations 1, 2, 6 and 7, chapter 6). These two characters alone prove to be a more sensitive indicator of taxonomic status than either HI scores or multivariate analysis of external morphology. Unfortunately, neither are visible in x-ray images (pers. obs. using *X. clivii* and *X. largeni*), necessitating the sacrifice of specimens for taxonomic determination by this means.

Univariate analysis of variance on data for these two characters showed non-overlapping ranges for *X. muelleri* and *X. l. laevis*. The mean ratio of inter-otic distance over cranium width was determined for both species; this value for *X. laevis* is 0.22 (range 0.16-0.27) and for *X. muelleri* 0.45 (range 0.36-0.51), and mean ratio of frontoparietal length anterior, to length posterior from pineal foramen for *X. laevis* is 0.34 (range 0.27-0.43) and for *X. muelleri* is 0.58 (range 0.49-0.65). The corresponding values for intermediates varied between mean values for each species.

This variation swamps the significant effects of sexual dimorphism for each of these characters: T=17,28, d.f.=11, p=0.000 for inter-otic gap [*X. laevis*, *X. muelleri*]; T=57,41, d.f.=11, p=0.000 for length of the frontoparietal posterior to the pineal foramen [*X. laevis*, *X. muelleri*]).

Osteological deformities

Introduction

An increased rate of osteological deformities is expected in hybrids and is consistent with the notion that hybridization causes genetic incompatibility and developmental instability (Littlejohn and Watson, 1973; Hewitt, 1988; Barton and Hewitt, 1989).

Materials and methods

Specimens (listed under the previous section) were assessed for osteological deformities. Each type of deformity, except for those believed to have occurred as a result of mechanical damage or regeneration, shall be given a reference number and addressed in turn.

Results

Deformities were found to be generally concentrated around the sacral portion of the vertebral column, often involving the presence of additional post-sacral vestigial processes (1). A number of specimens demonstrated expansion and occasional fusion of the last pre-sacral transverse process to sacral diapophyses (2), whilst in others pre-sacral vertebrae I and II were fused (3). Less common deformities included a short cultriform process (4), dwarfed septomaxillaries (5), irregular carpal lengths (6) and a straight anterolaterally directed first transverse process (7).

Species	Deformity code x frequency in sample	% deformed specimens
X. laevis (n=12)	1x1, 2x1, 3x1.	25%
X. muelleri (n=12)	1x4, 4x1	42%
Intermediate (n=9)	1x1, 3x1, 4x1, 5x1, 6x1, 7x1	67%

Table 7-4

Summary of prevalence of different forms of osteological deformity for each group.

Table 7-4 summarizes the frequency distribution of osteological aberrations in each of the examined samples. Deformity rates are high in all three groups, but highest and most variable for intermediates.

Mate-call

Introduction

Whilst widely recognized as the primary means of pre-mating isolation in amphibians, the species-specific mate-call has been shown, in *Xenopus*, to be weak in this respect, especially in areas of sympatry (Tinsley and Kobel, 1996). It has been known for the mate-call to be by-passed as a pre-mating barrier in crowded ponds where males might display indiscriminate mate-clasping behaviour (Picker, Harrison and Wallace, 1996), leading to a breakdown in positive-assortive mating. Mate-calls were recorded from a range of specimens, chosen on account of HI scores and results from multivariate analysis of external morphology in an attempt to identify evidence of hybridization.

Materials

Calls were recorded from lab-maintained, male *Xenopus* brought back live from the field. Since it was not known whether specimens identified as *X. l. laevis* collected during our field-work had previously been contaminated through introgression, a sample of 8 *X. l. laevis* collected by R. Tinsley in 1998 from the Cape were used for comparison with 3 specimens collected from Pretoriuskop, Kruger National Park. The calls of Cape *X. l. laevis* have previously been shown not to differ from those collected locally from Mpumalanga, approximately 50km from our study site (Fischer *et al.*, 2000).

Only local *X. muelleri* collected from Nkambeni, Swaziland were available for the present study. A previous study, however found no significant difference between the mate-calls of local and Tanzanian *X. muelleri* (Fischer *et al.*, 2000).

A number of sites contained specimens of intermediate morphology. Recordings were consequently made of all 'compliant' calling males from these sites. Calls were also successfully recorded from a sample of 2 lab-bred F1 *X. laevis* (female) x *X. muelleri* (male) hybrids.

Methods

Each male was stimulated with 100 IU of human chorionic gonadotrophin, administered by injection on the morning preceding night-time recordings. In the event of males not calling on first or second nights, this was followed by up to two more injections, as necessary, administered at 48 hourly intervals.

Individual specimens were placed in 40 x 25cm transparent containers. Calling males were transferred in turn to a large, lightproof, circular water container (60cm wide, 80cm deep) used for recording, and containing a single hydrophone, positioned mid-depth on the inside surface. Water temperature was maintained at 22°C on each recording night.

The hydrophone, a Dowty SSQ904, had a peak sensitivity of 174dB (re. 1V/uPa) and frequency response ranging from 10Hz to 5kHz (±3db). A custom-built power circuit supplied power

to the hydrophone; calls were recorded, amplified and stored on TDK DA-RXG DAT 120 tapes, using a SONY DAT recorder for analysis.

Mate-call analysis generally followed the methods of Parsons and Jones (2000). Calls were digitized to computer using BatSound software (Pettersson Elektronik AB, Uppsala, Sweden) at a sampling rate of 44.1kHz (effective rate 441kHz), using a standard soundcard. Calls with highest signal-to-noise ratio were chosen for analysis in real-time. A mid-pass (9-10.5kHz), 8th order Butterworth filter was applied to the signal. Inter-click intervals were obtained using pulse interval analysis, and employed a detection delay of 1ms and detection threshold values ranging from 4-15%. Results were crosschecked by eye against sonograms to ensure that there were no outliers.

Results

Calls were recorded from 5 local *X. muelleri*, 3 local *X. l. laevis*, 8 Cape *X. l. laevis*, 2 lab-raised F1 hybrids and 10 specimens of intermediate morphology. All calls were examined aurally, visually and subjected to temporal analysis. Data for two temporal components of each call were extracted for inter-specific statistical comparison, inter-click interval and call rate.

Comparison with recordings from the literature

Although the qualitative characteristics of *X. muelleri* calls closely resembled those reported in the literature, a small degree of temporal variation was apparent (see Fischer *et al.*, 2000). This variation was attributed to a difference of 4°C in recording temperature between studies, a factor known to affect the temporal characteristics of amphibian mate-calls (Bellis, 1957; Vigny, 1979; Stock, 1998).

In contrast, the calls of local *X. l. laevis* differed both qualitatively and quantitatively from those of Cape specimens and *X. l. laevis* reported in the literature (Vigny, 1979; Picker, 1980; Fischer *et al.*, 2000), suggesting that the genetic effects of introgression persist even in the absence of morphological evidence of genetic contamination. Calls of local *X. l. laevis* will therefore be discussed with those of other specimens of intermediate morphology (see below).

Visual and aural comparison of calls

Audio and visual comparisons of *X. l. laevis* (Cape) and *X. muelleri* (local) calls immediately revealed considerable qualitative differences. The calls of *X. l. laevis* consist of sound pulses, separated by three distinct classes of inter-pulse interval that are delivered according to a stereotyped temporal pattern. It would appear that each component of the call is subject to individual amplitude-modulation, whilst inter-click interval remains remarkably constant for each component of the call.

Previously, only two components of the *X. l. laevis* call have been described, a slow followed by fast click-repetition rate (see Vigny, 1979; Picker, 1980; Fischer *et al.*, 2000). A third, slower rate, not previously reported for *X. l. laevis*, has been observed for each of the calls recorded from Cape specimens, and immediately follows the fastest click-repetition rate, making the stereotype temporal

sequence medium-fast-slow (Figure 7-6, Figure 7-7, Figure 7-8). On average, call-unit rate was 1.1/sec (range 0.9-1.2/sec, s.d.=0.11). Males recorded in the lab called continuously for up to 20 minutes at a time.

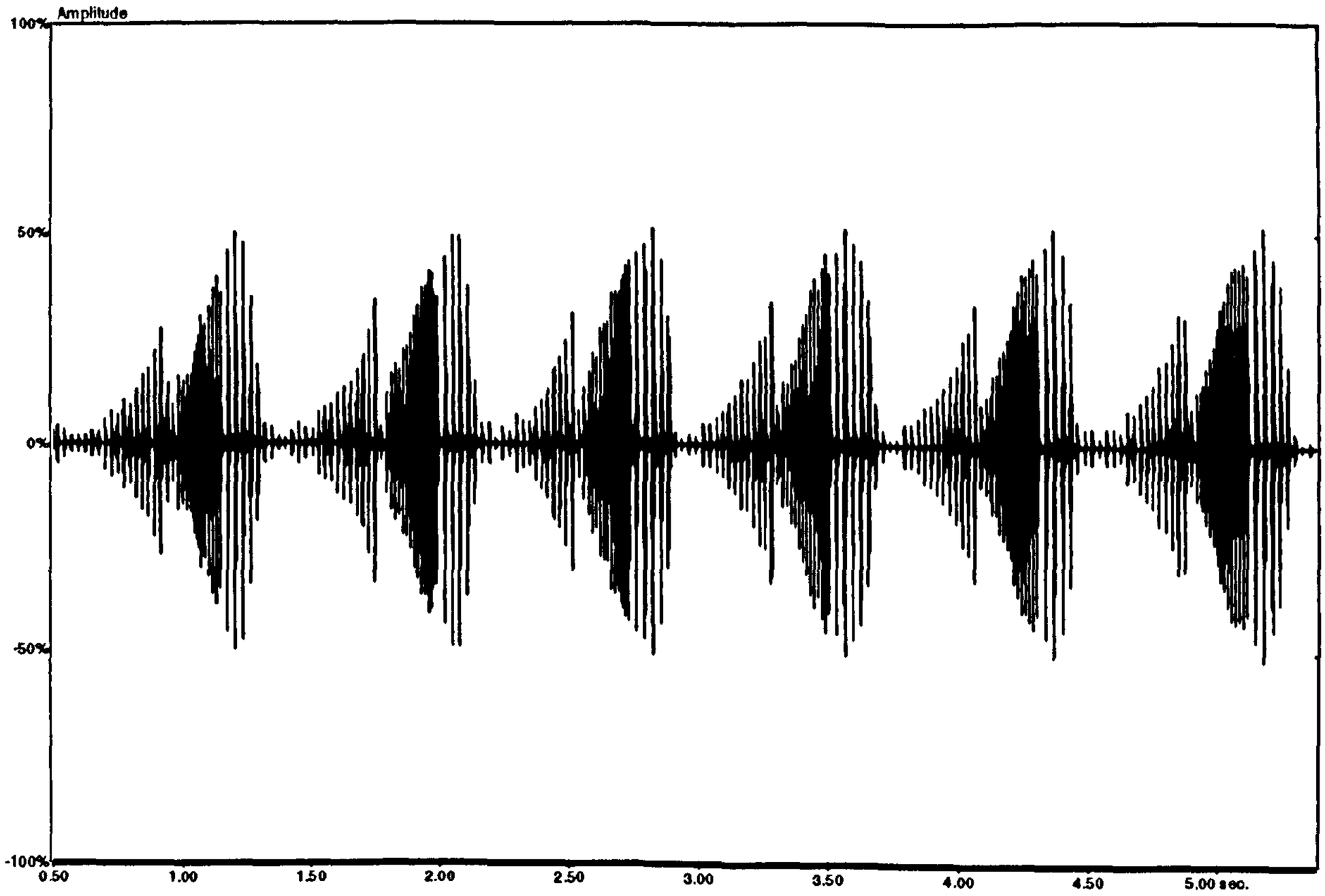


Figure 7-6

Oscillogram of a *X. l. laevis* mating-call showing inter-click interval and relative amplitude of call components over a 5 second period.

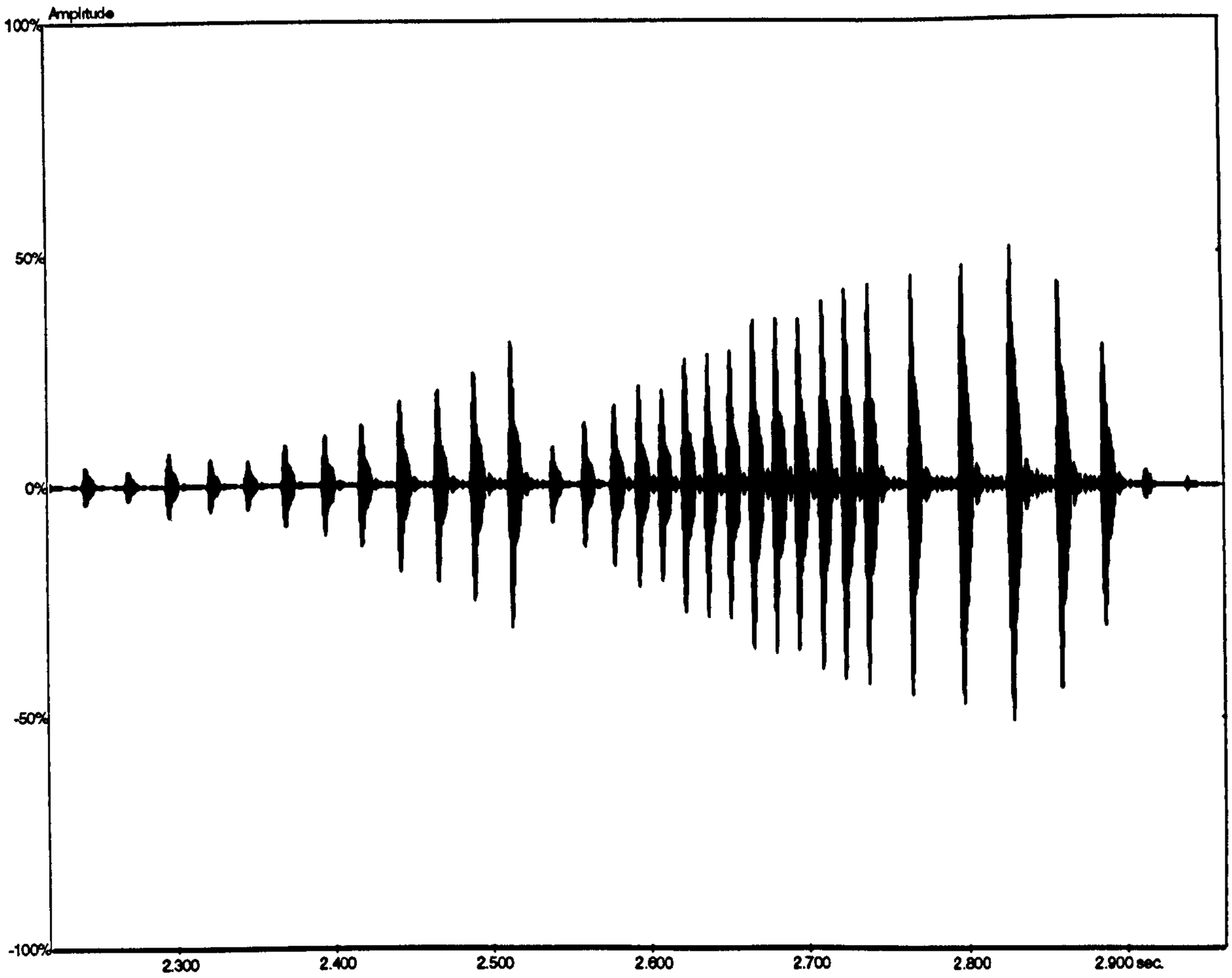


Figure 7-7

Oscillogram showing inter-click interval and relative amplitude of call components of a single *X. l. laevis* call-unit. Note three phases of call: medium-fast-slow click-repetition rates.

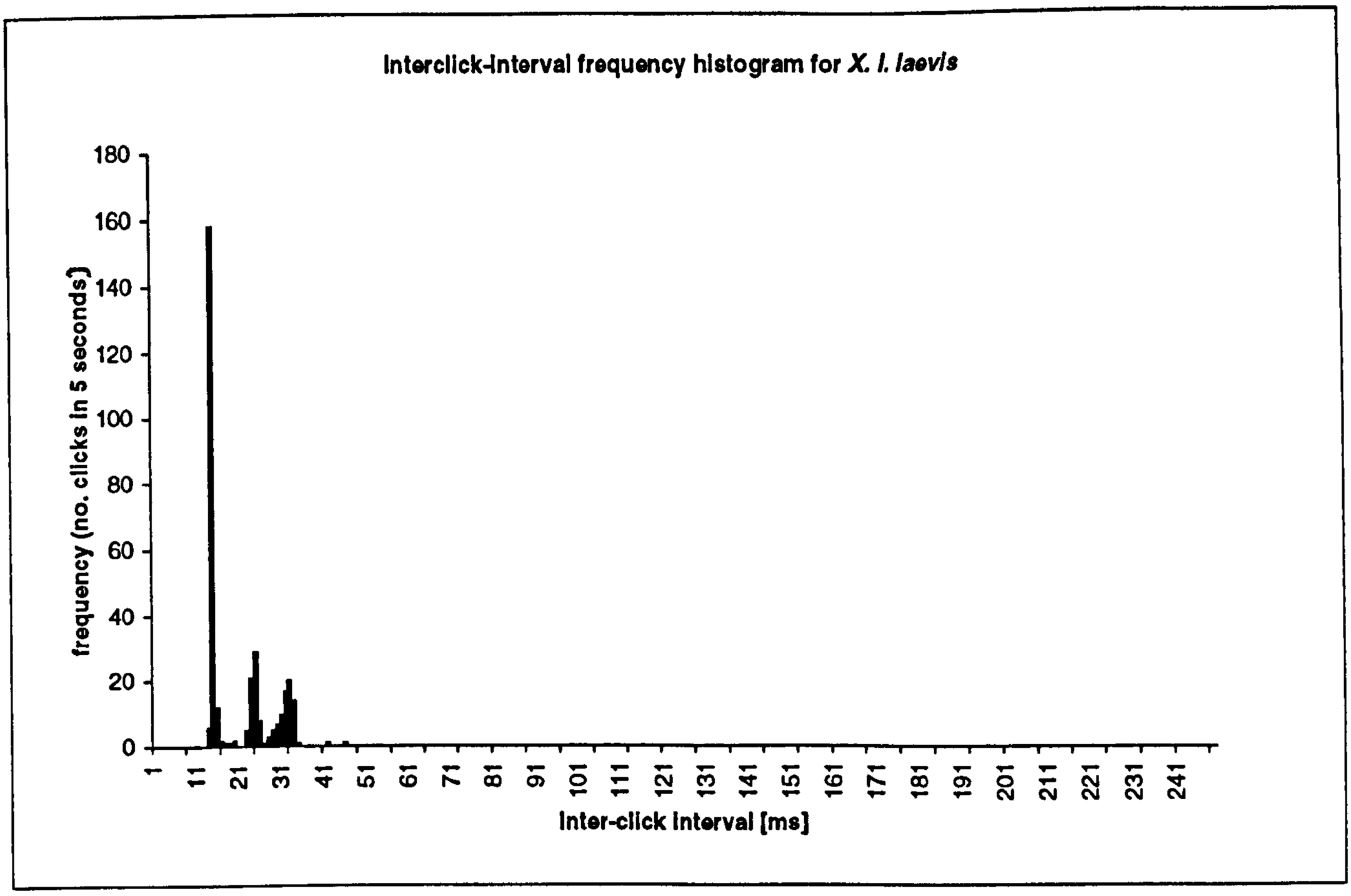


Figure 7-8

Representative inter-click interval histogram for a single specimen of *X. l. laevis* showing frequency distribution of different classes of inter-click interval over a five second period.

In contrast, the calls of *X. muelleri* are less complex, and consist of a continuous series of double (occasionally single or triple) clicks in quick succession (Figure 7-9). In real time, double clicks sound as one single 'tick', discernable as two only upon visual inspection of the sonogram. Each double click is separated by a constant-length, short inter-click interval; the second click is usually the louder of the two. Each double click unit, in turn, is separated from the next pair by a longer, relatively constant-length interval (Figure 7-10). Mean call-unit rate was 5.3/sec (range 4.4-6/sec, s.d.=0.59). The duration of male calls varied from a 10 seconds to 15 minutes, repeated regularly throughout the night.

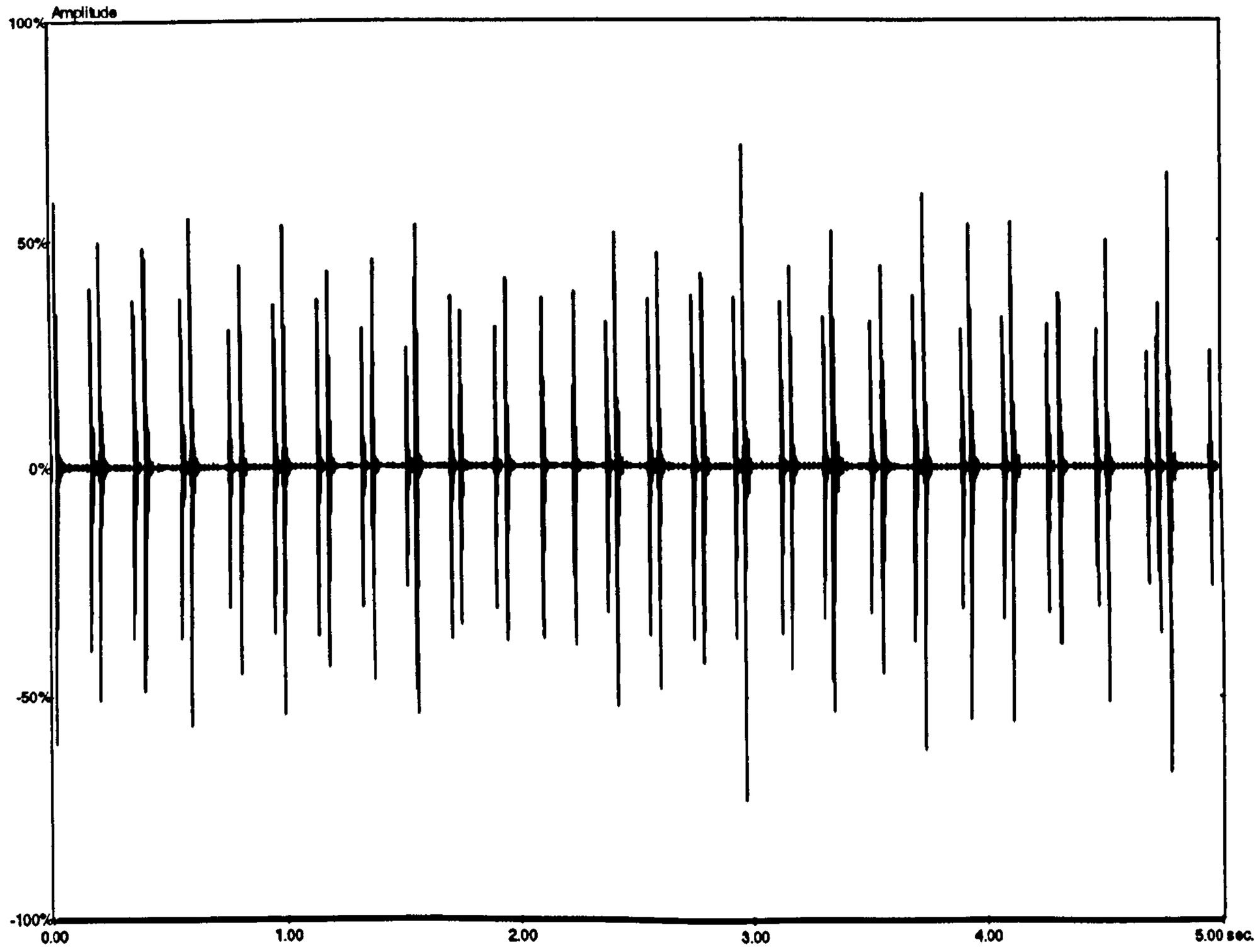


Figure 7-9

Oscillogram of a *X. muelleri* mating-call showing inter-click interval and relative amplitude of call components over a 5 second period.

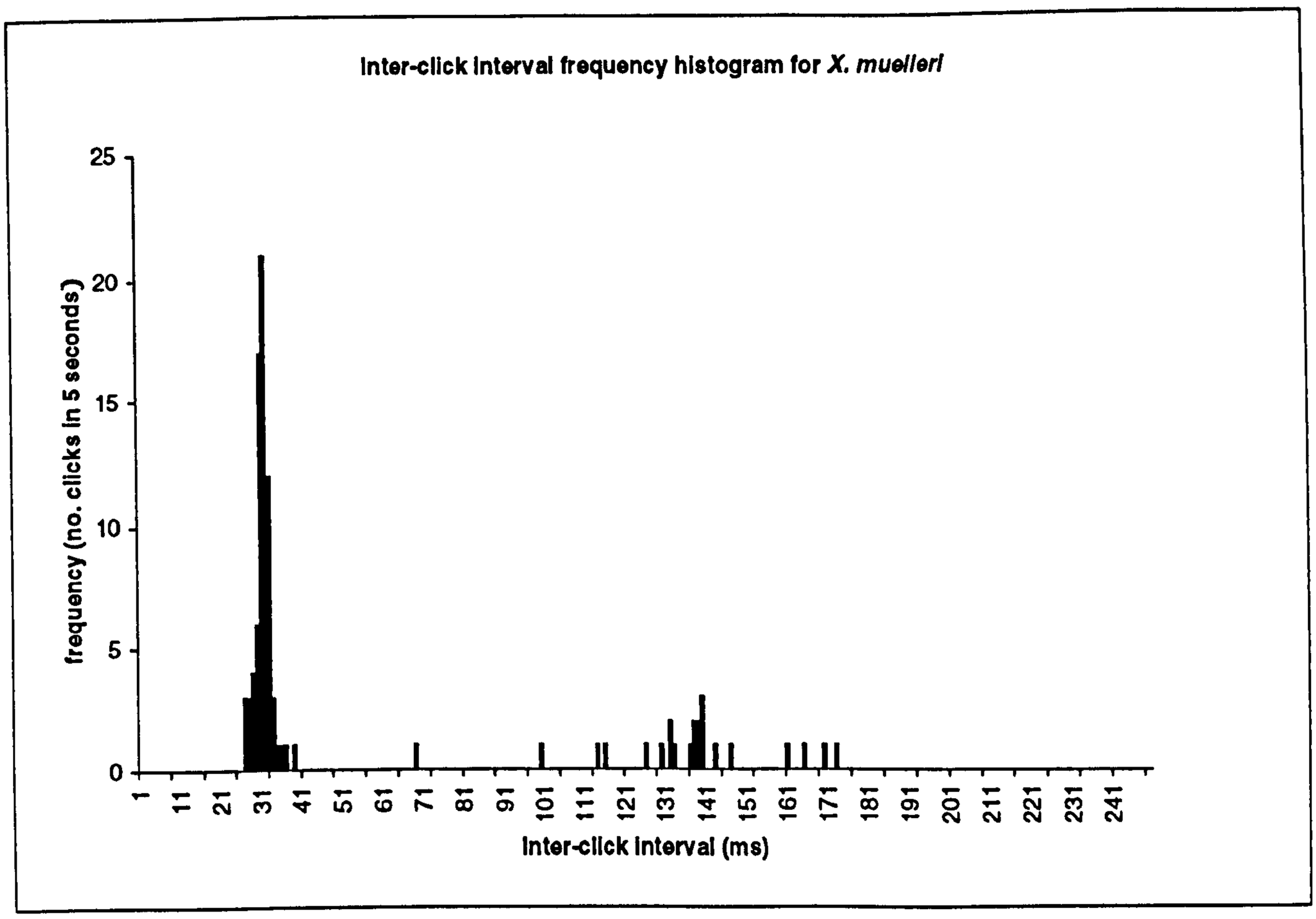


Figure 7-10

Representative inter-click interval histogram for a single *X. muelleri* specimen showing frequency distribution of different classes of inter-click interval over a five second period.

Calls of lab-produced F1 hybrids differed from those of both pure species and from one another. Qualitatively, calls resembled those of *X. muelleri* more closely than *X. laevis*. The call of F1-1 consisted of a series of phrases of 4-5 pulses, each pulse separated from the other by short, irregularly spaced, inter-pulse intervals, and each pulse group from the next by a longer interval (Figure 7-11). Pulses within each group increased sequentially in amplitude. The call of F1-2 was composed of a series of phrases of 1-4 pulses, arranged in the same manner as F1-1, but with more variable amplitude modulation within each pulse of calls (Figure 7-12). Inter-click interval was, on the whole, more variable than parent taxa (Figure 7-13). Mean call-unit repetition rate was 3.4/sec (range 2.6-4.2/sec, s.d.=1.13).

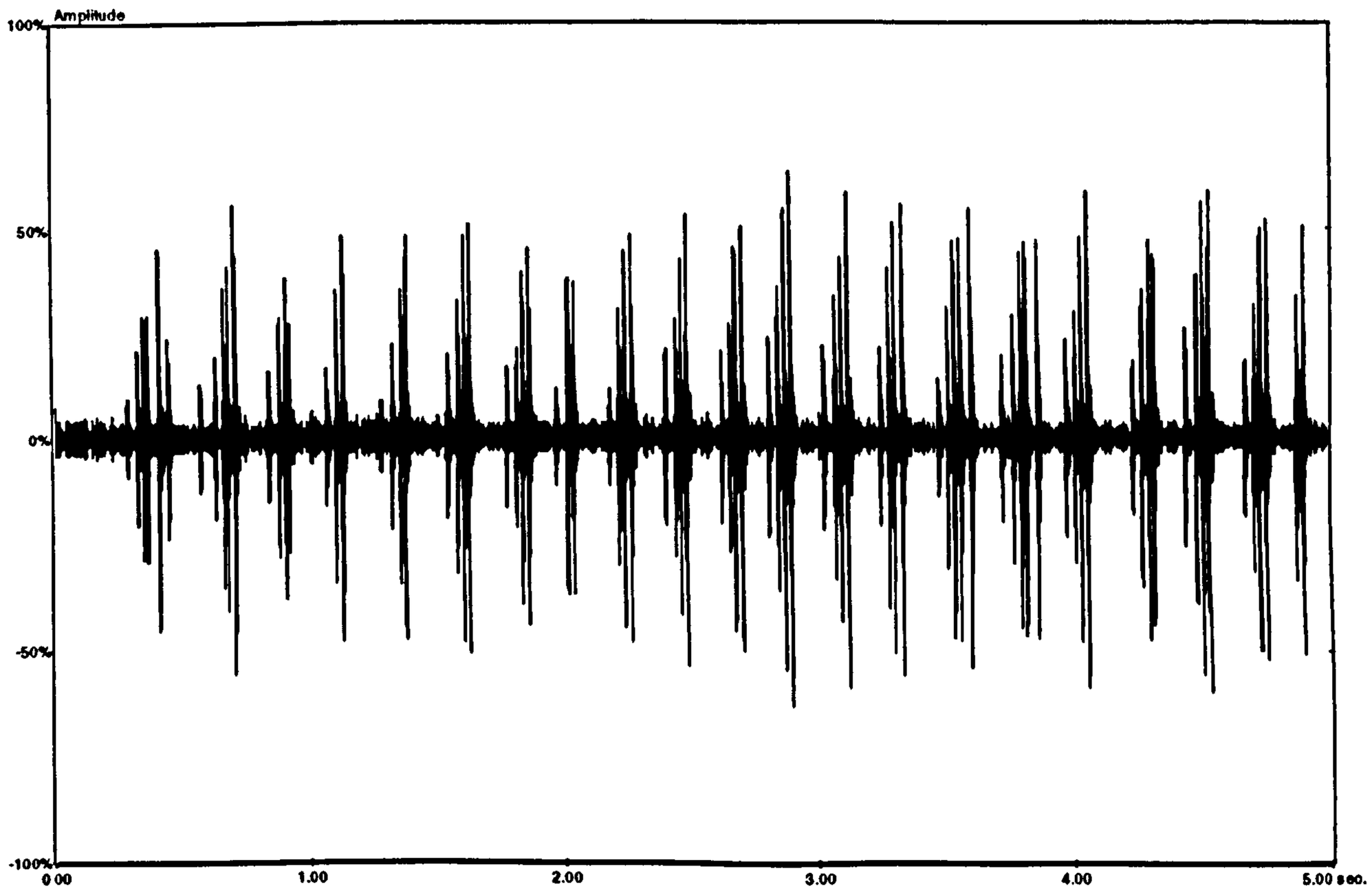


Figure 7-11

Oscillogram of hybrid (F1-1) mating-call showing inter-click interval and relative amplitude of call components over a 5 second period.

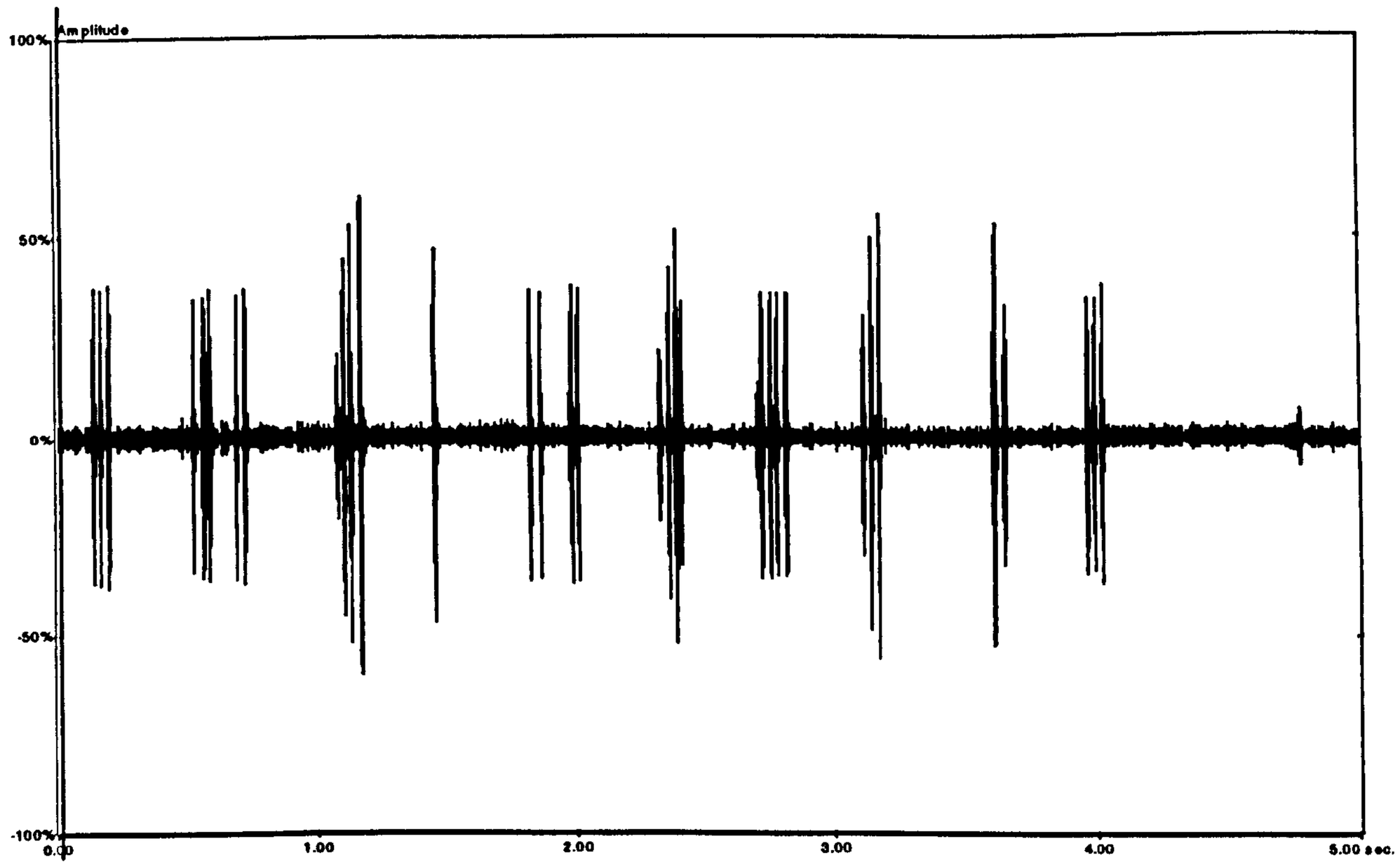


Figure 7-12

Oscillogram of hybrid (F1-2) mating-call showing inter-click interval and relative amplitude of call components over a 5 second period.

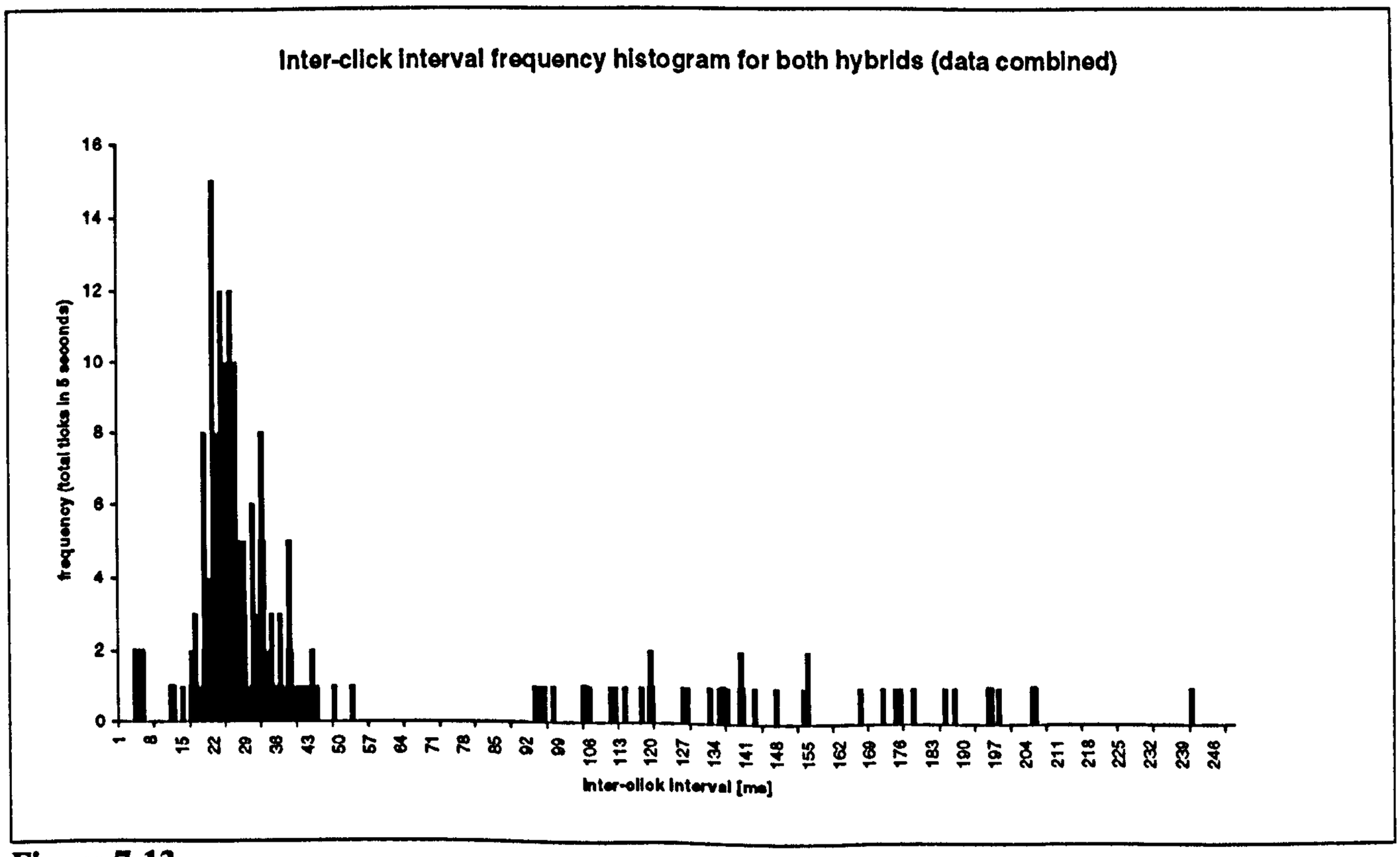


Figure 7-13

Inter-click interval histogram hybrids (combined data) showing frequency distribution of different classes of inter-click interval over a five second period.

Calls of intermediates varied considerably, ranging from an F1 hybrid-like call (Figure 7-14) to a call that was almost pure *X. laevis*-like (Figure 7-15). The F1 hybrid-like call (Figure 7-14) was characterized by longer trains of pulses per phrase (4-16) than either F1 hybrid, whilst the *X. laevis*-like call (Figure 7-15) differed from *X. laevis* only in having a call that was temporally drawn out. The calls of most specimens of intermediate morphology, including those previously considered to have been local *X. l. laevis* but found later to have intermediate mate-calls, comprised of two components, a fast and slow click repetition-rate, showing an irregular temporal pattern, each pulse group separated from the next by inter-pulse intervals of varying lengths, some showing regular amplitude modulation (Figure 7-16, Figure 7-17, Figure 7-18, Figure 7-19, Figure 7-20). These calls had a faster repetition rate than those of Cape *X. l. laevis*. Call-unit repetition rate for all intermediates varied from 1-2.4/sec (mean=1.6/sec, s.d.= 0.38).

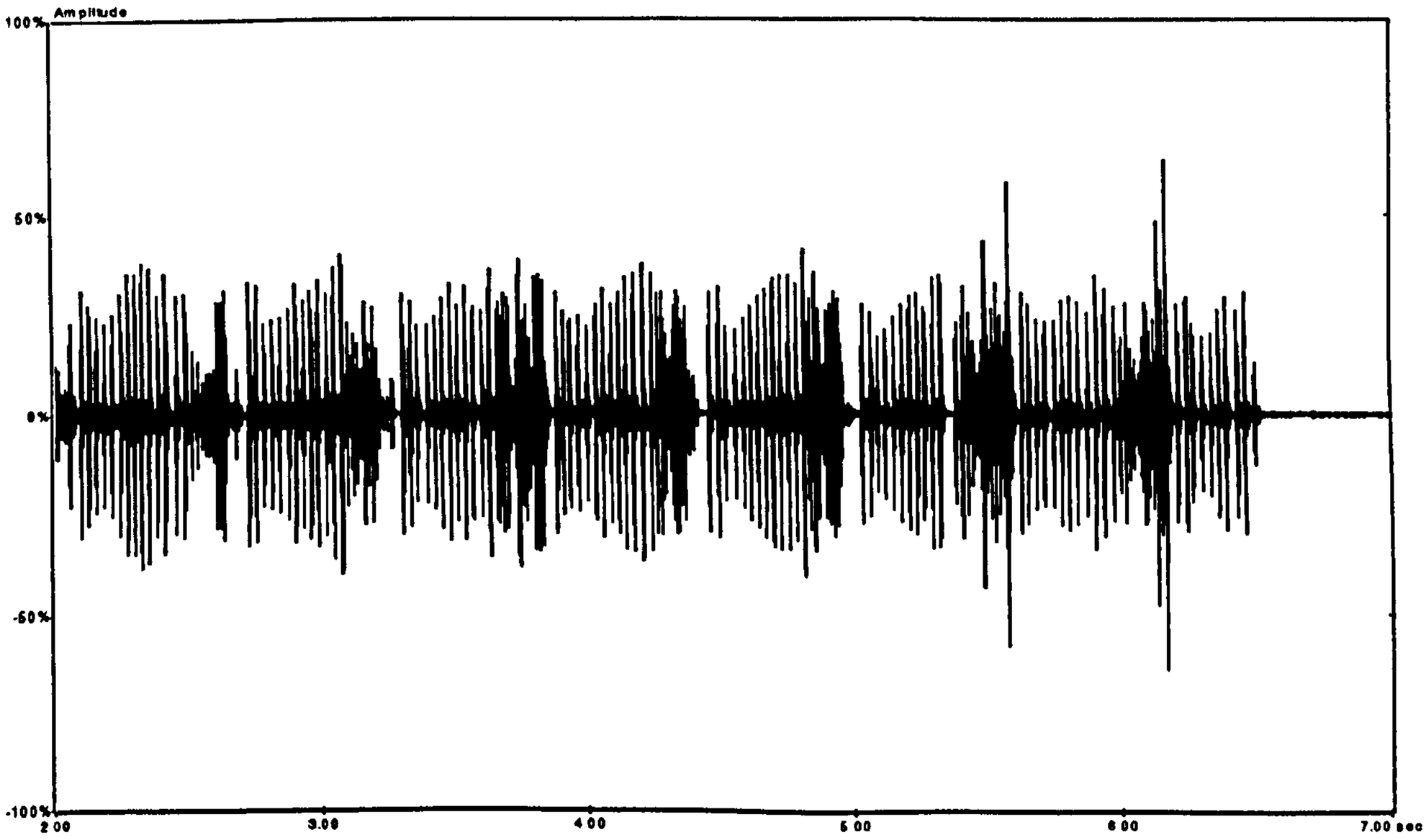


Figure 7-14

Oscillogram of intermediate (Int. 1) mating-call showing inter-click interval and relative amplitude of call components over a 5 second period.

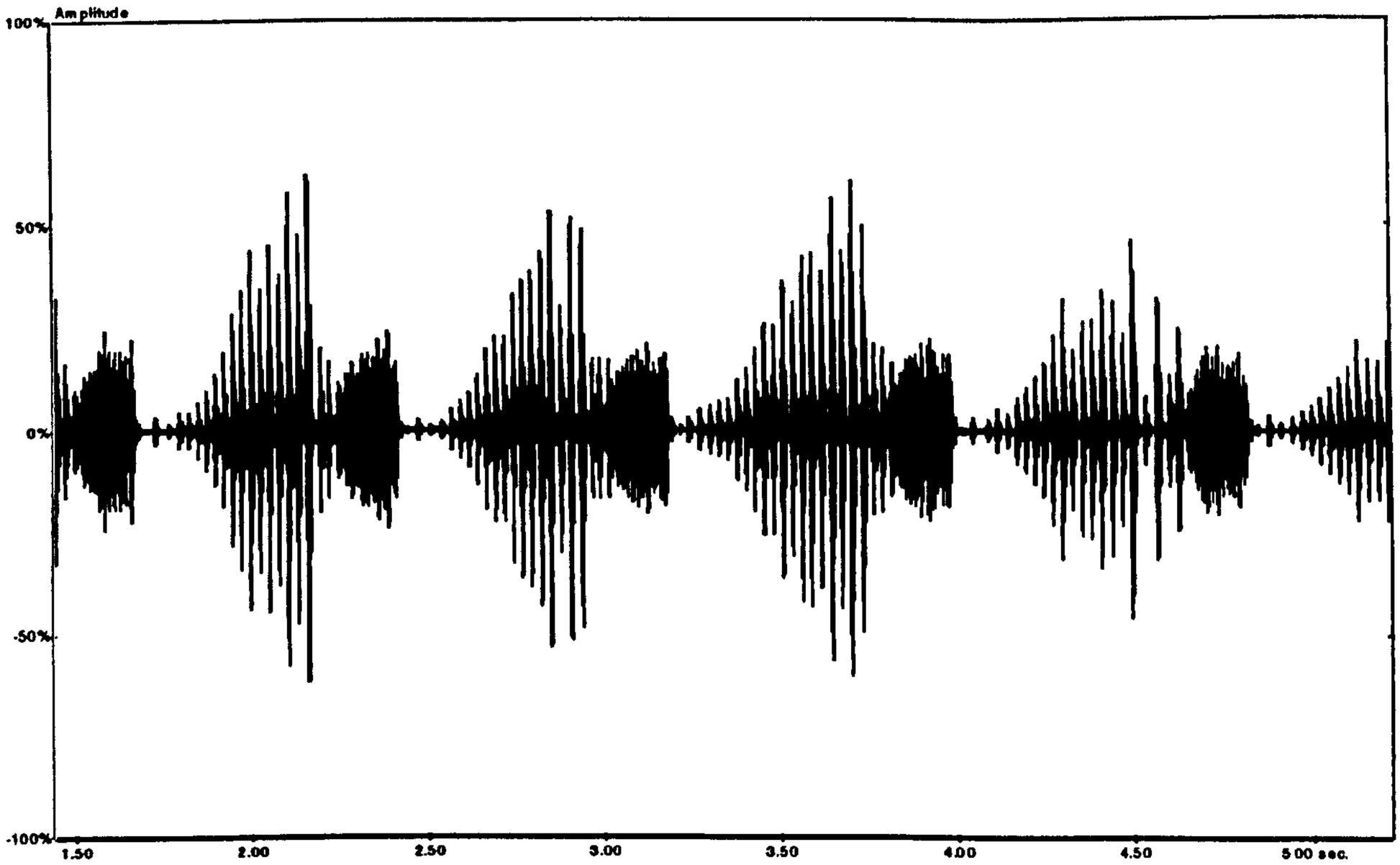


Figure 7-15

Oscillogram of intermediate (Int. 28) mating-call showing inter-click interval and relative amplitude of call components over a 5 second period.

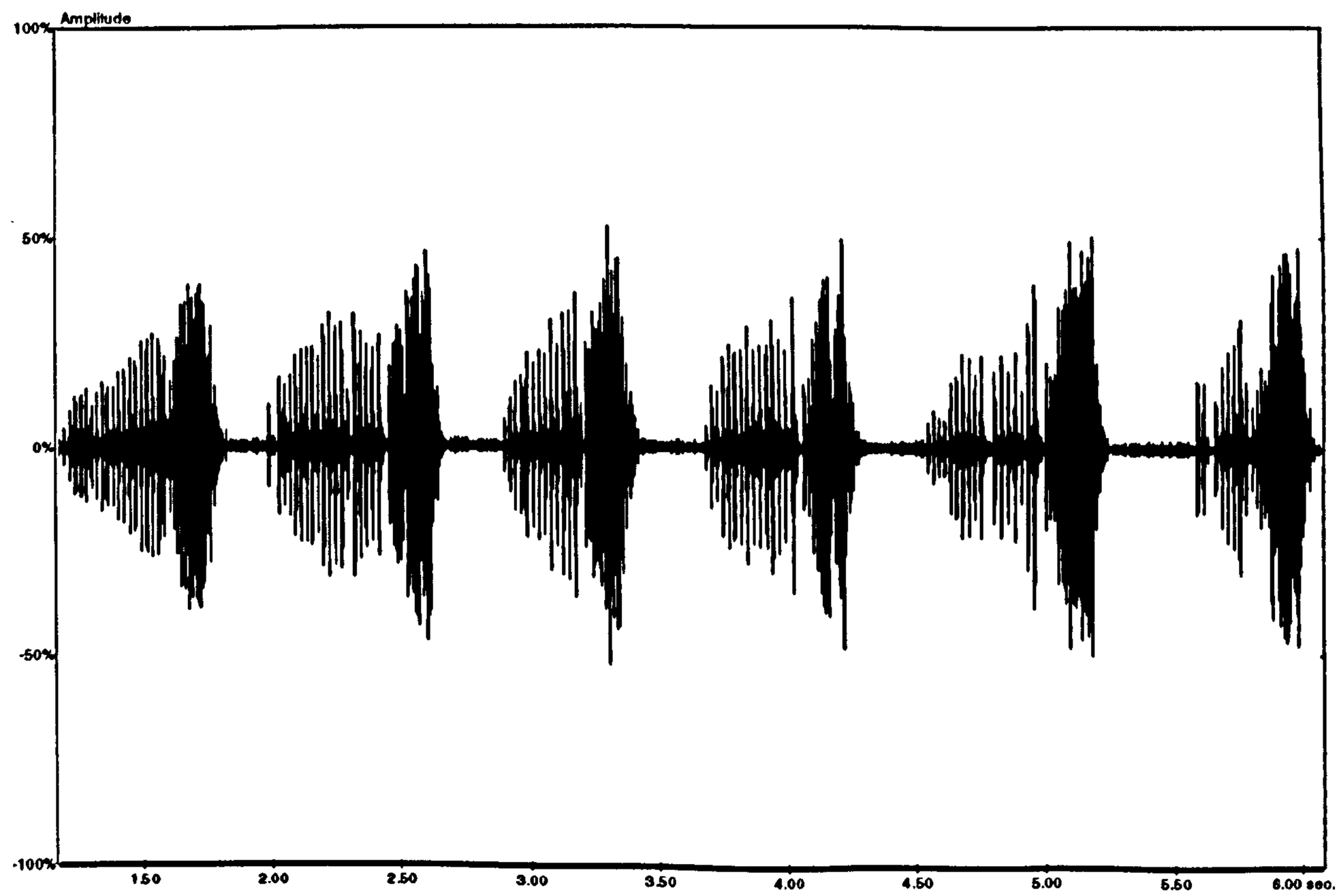


Figure 7-16

Oscillogram of intermediate (Int. 25) mating-call showing inter-click interval and relative amplitude of call components over a 5 second period.

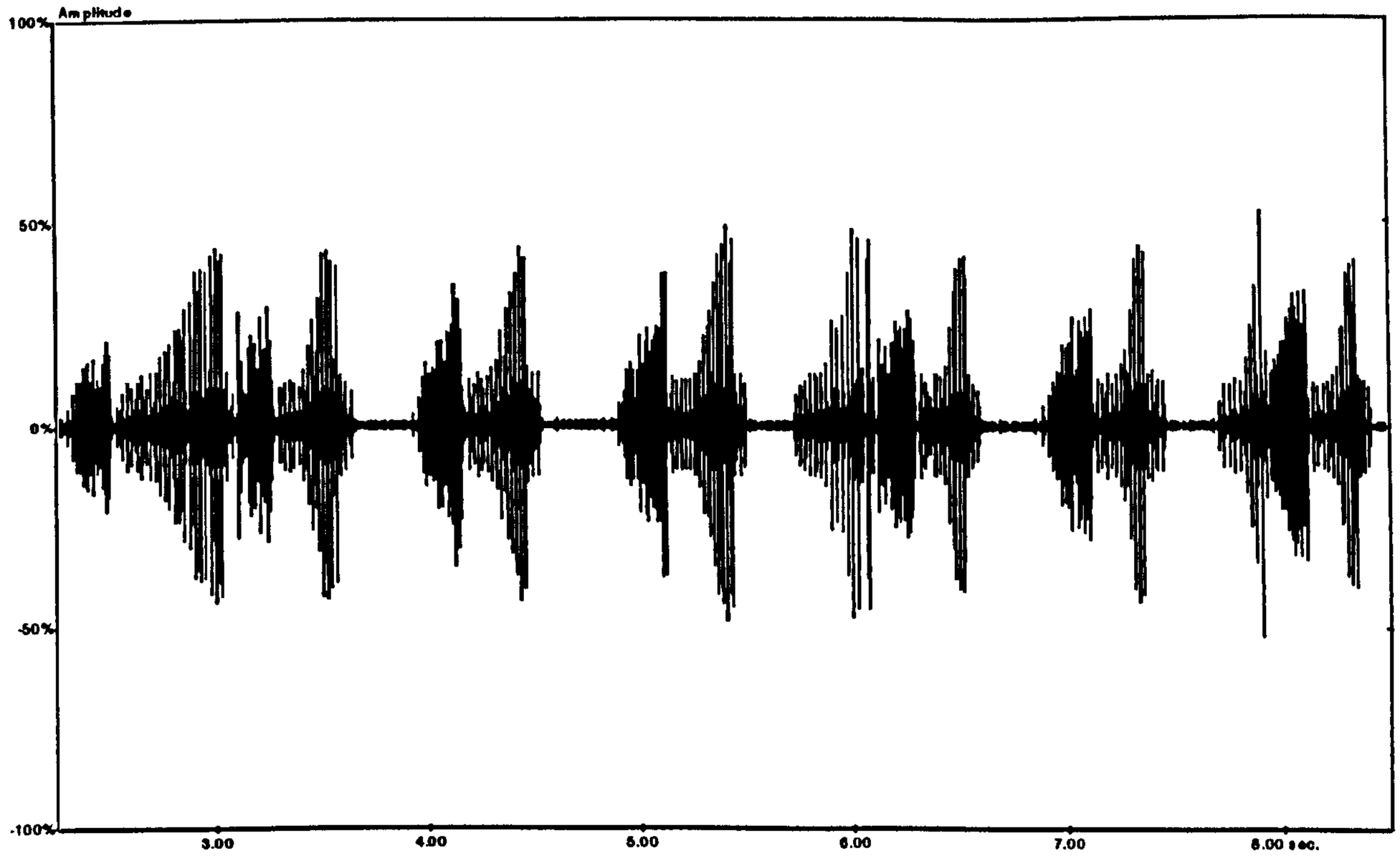


Figure 7-17

Oscillogram of intermediate (Int. 4) mating-call showing inter-click interval and relative amplitude of call components over a 5 second period.

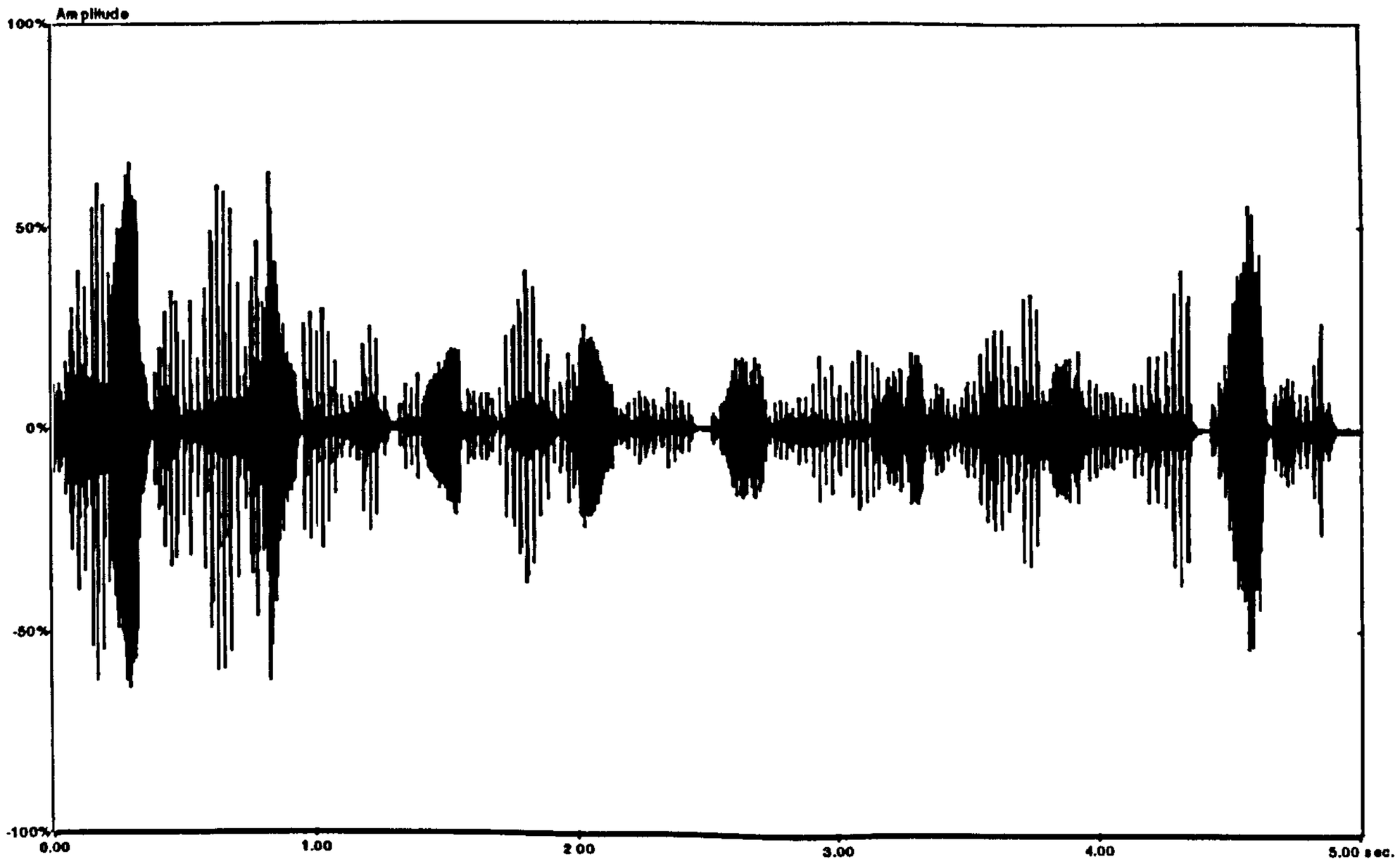


Figure 7-18

Oscillogram of intermediate (Int. 45) mating-call showing inter-click interval and relative amplitude of call components over a 5 second period.

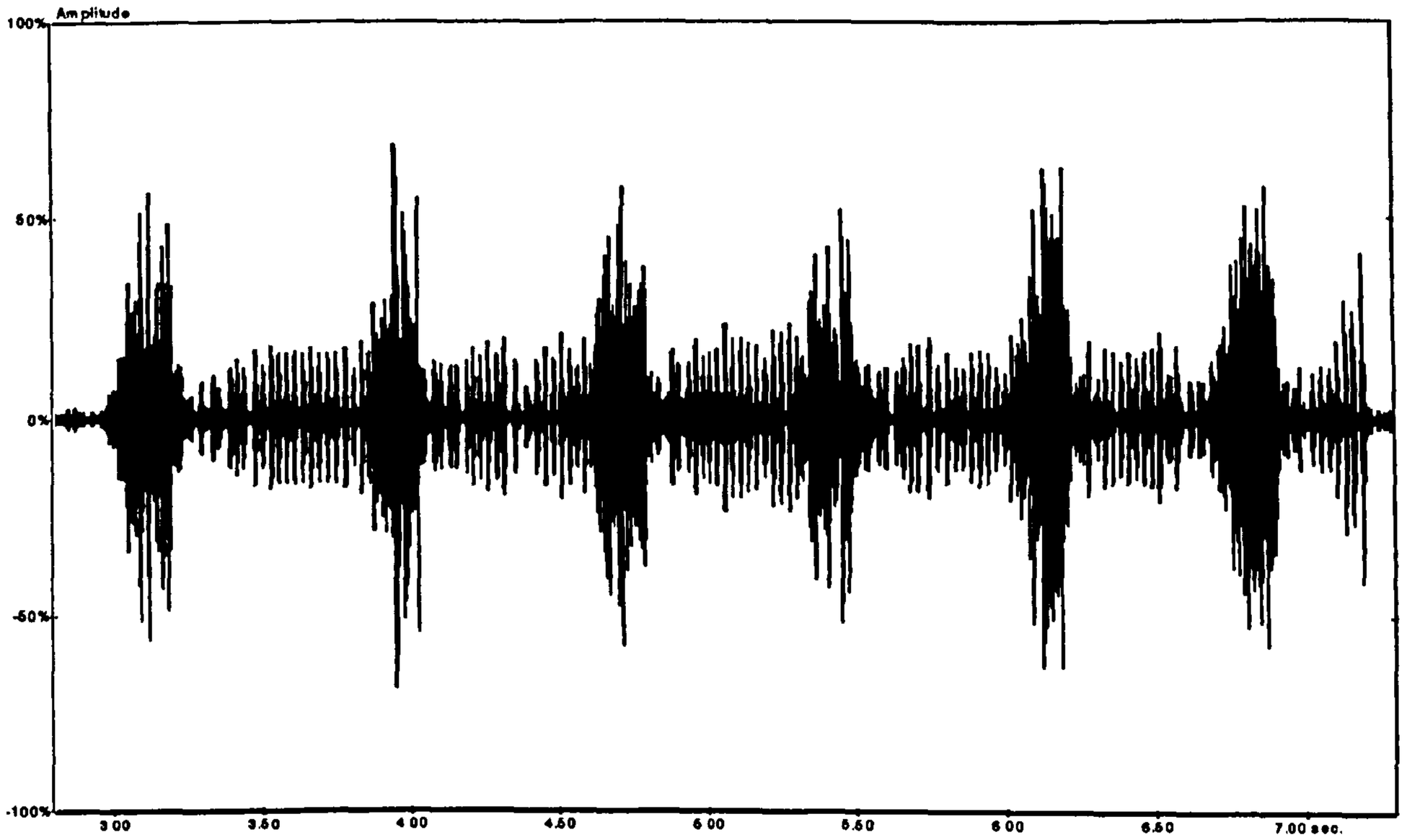


Figure 7-19

Oscillogram of intermediate (Int. 8) mating-call showing inter-click interval and relative amplitude of call components over a 5 second period.

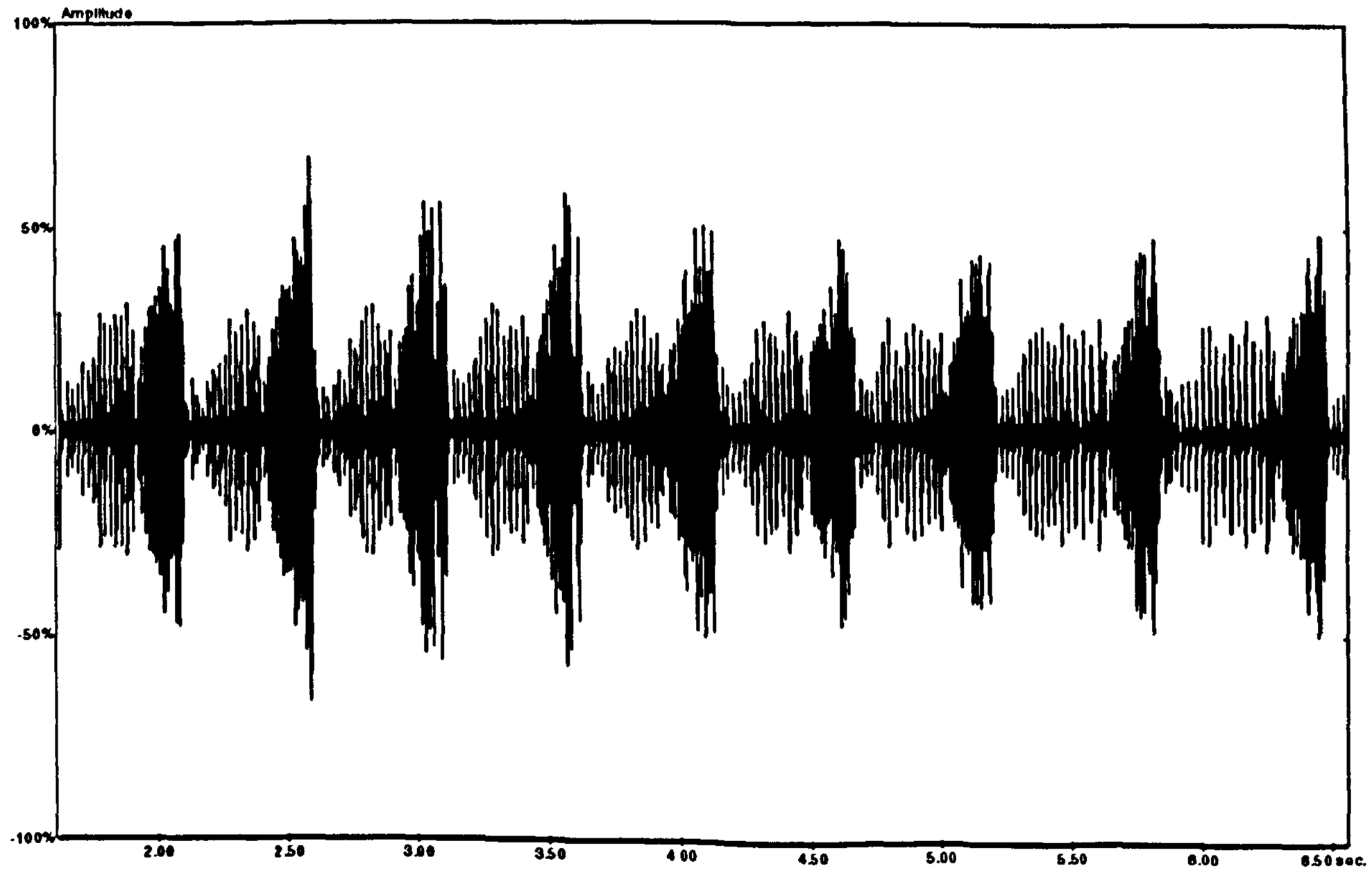


Figure 7-20

Oscillogram of intermediate (Int. 9) mating-call showing inter-click interval and relative amplitude of call components over a 5 second period.

Statistical analysis of mate-calls

Call-unit repetition rate and inter-click interval were measured for each calling individual. Peak interval duration for *X. laevis* (Cape) was constant at 15ms, and for *X. muelleri*, more variable, at around 31ms. Mean call-unit repetition rate was 1.1/sec for *X. laevis* and 4.4/sec for *X. muelleri*. Although data for these two components were non-overlapping for *X. muelleri* and *X. laevis*, variables were nevertheless analyzed by DFA, using species as a grouping variable. The analysis maximally separated *X. muelleri* from *X. laevis*, and gave each intermediate a statistical value corresponding to its position relative to each pure species, based on these two call parameters. All fell between the minimum DFA score for *X. muelleri* and maximum score for *X. laevis* (Figure 7-21), providing the clearest indication yet that hybridization is occurring naturally in the field. Furthermore, comparison of F1-hybrid call parameters with those of wild caught intermediates clearly shows the calls of all intermediates to be more *X. l. laevis* than *X. muelleri*-like. Rather than indicating asymmetrical introgression, this is consistent with HI scores for all recorded male intermediates, which lie in the *X. l. laevis* end of the HI score range. Male intermediates with HI scores in the *X. muelleri* x F1 range were non-compliant.

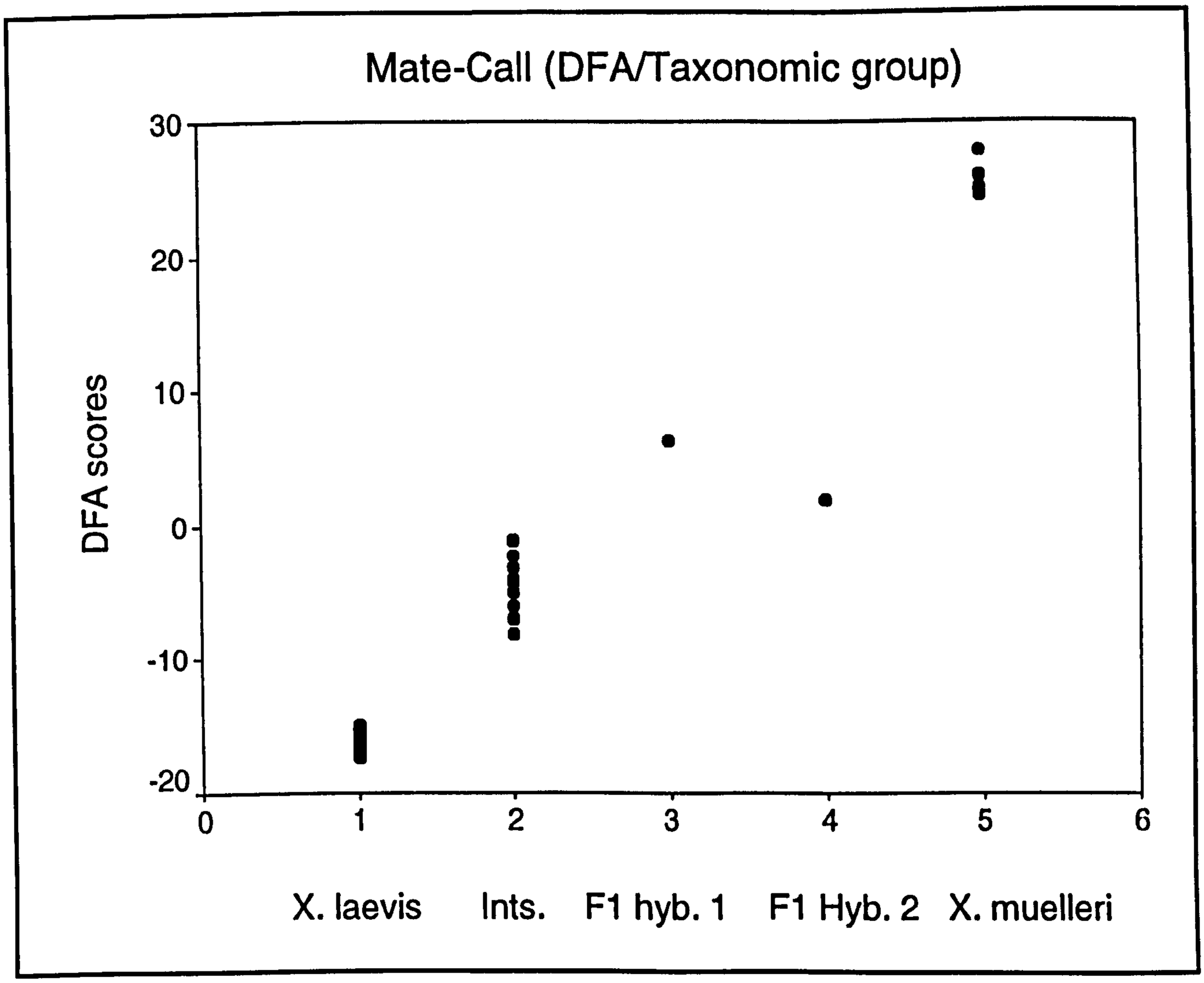


Figure 7-21

Scatter plots of DFA scores of mate-call statistical components. 1-*X. laevis*, 2- intermediates; 3&4-F1-1, F1-2 respectively and 5-*X. muelleri*.

A strong positive correlation (Pearson's correlation, 0.95) exists between HI scores of external morphology and DFA of mate call parameters (Regression r^2=91.1, F=266), as is shown in Figure 7-22. Although when averaged over the whole sample, this correlation is high, the relationship between call parameters of intermediates and their respective HI score is lower, indicating an uncoupling of the genes involved in determination of morphology and mate-call.

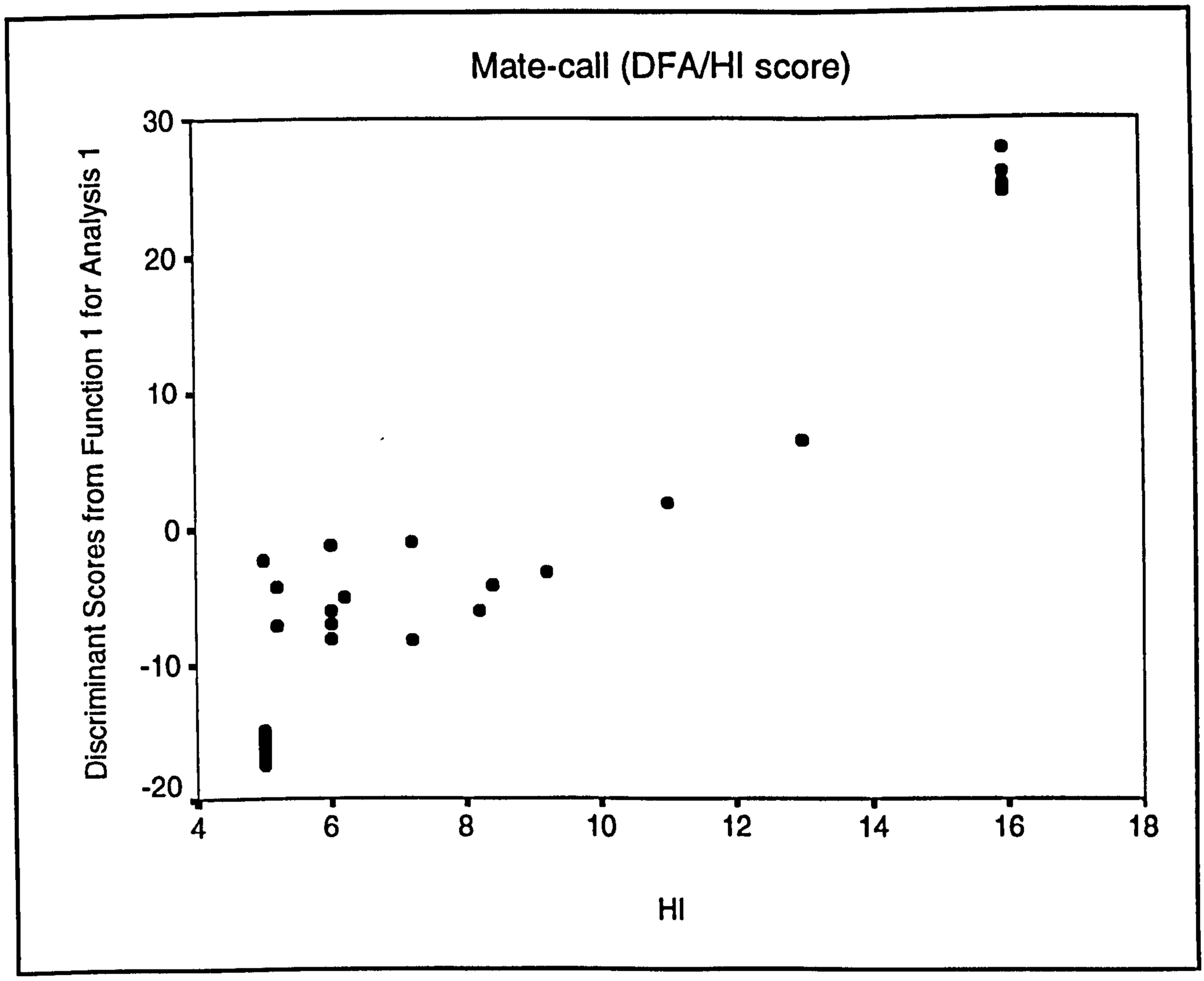


Figure 7-22

DFA mate-call inter-click interval data plotted against HI score for *X. muelleri*, *X. l. laevis*, intermediates and F1 hybrids (x2). All pure *X. laevis* cluster in the lower left hand corner of the scatter plot, each having an HI score of 5. *Xenopus muelleri* in contrast, scatter in the top right hand corner of the plot, each having an HI score of 16. F1 hybrids have HI scores of 11 and 13. Mate-call data place each intermediate more closely to *X. laevis* than to *X. muelleri*, in accordance with their HI scores.

Albumin protein gel electrophoresis

Introduction

Xenopus muelleri and *X. l. laevis* both possess a pair of albumin proteins, each said to be the translational product of two distinct mRNAs (Westley, Wyler, Ryffel and Weber, 1981). Isoproteins in *X. muelleri* have a molecular weight of 70kDa, whilst the molecular weights of albumin proteins in *X. laevis* are 70 and 74kDa. Previous workers have demonstrated a high degree of inter-specific variation in the mobility of these proteins (Bisbee, Baker and Wilson, 1977; Graf and Fischberg, 1986; Fischer *et al.*, 2000). This property was used to distinguish these two species apart.

Materials

In addition to specimens returned live to the lab from 1999 fieldwork, Cape *X. l. laevis* collected in 1998, and *X. muelleri* collected from Ndumu, southeastern Africa in 1995 were also incorporated into this study for comparison between local and non-locally collected samples.

Methods

Each individual was anaesthetized by immersion in MS222 and bled by venipuncture on the dorsal surface of the tarsus. Preparation of blood samples followed the methods of Laemmli (1970): Coagulation of each 50µl blood sample was prevented by dilution with 1:10 with PBS/EDTA (PBS 0.2% (w/v) EDTA 7.3). The sample was centrifuged, and the supernatant diluted 1:100 (v/v) with PBS/0.2% (v/m) EDTA prior to dilution 1:1 (v/v) with sample buffer (Laemmli, 1970; Fischer *et al.* 2000). Samples were heated to 80°C for 20 minutes. A sample of 10µl from each of these was pipetted into each sample well of an 8 x 7cm sodium dodecyl sulphate poly-acrylamide gel (SDS-PAGE). Albumin proteins were separated by exposing gels to a 30mA current, at various voltages (100-140v) for up to an hour. Gels were subsequently stained for up to 30 minutes in 0.1% Coomassie blue R-250 in fixative (40%MeOH, 10% HOAc) and counter-stained in 40%MeOH/10%HOAc. Stacking and separation SDS-PAGE gels were made according to the methods of Laemmli (1970). Calibration of protein molecular weights was achieved using SDS-PAGE high molecular weight standards, ranging from 14,300 to 220,000 in molecular weight.

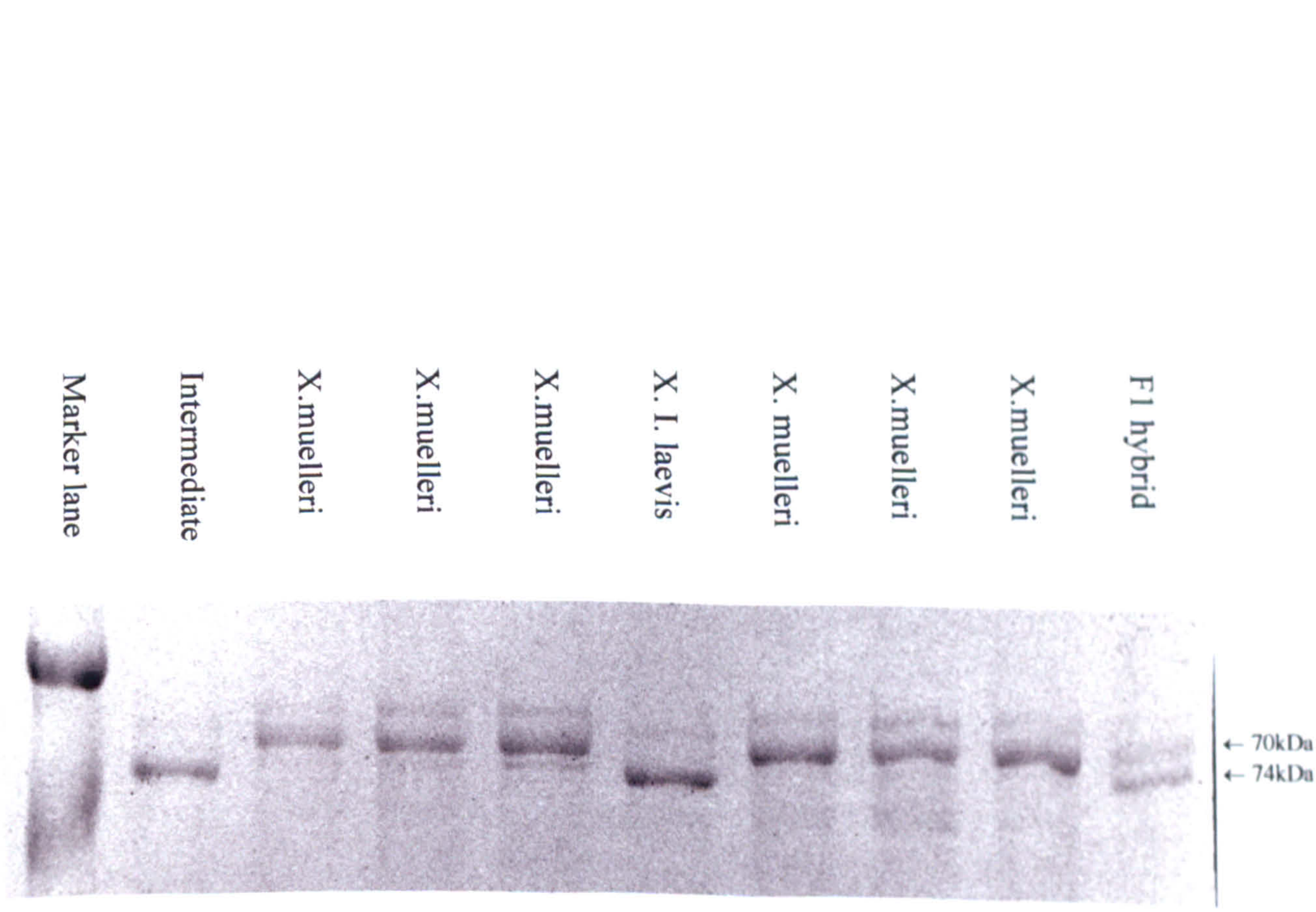
Results

Gel electrophoresis was carried out on the serum of 37 specimens. These included two lab raised F1 hybrids (male *X. muelleri*, female *X. l. laevis* parents) and an F2 backcross (hybrid [as for F1] backcrossed with male *X. muelleri*), two non-local *X. muelleri* and a further 2 non-local *X. l. laevis*. Thirty further specimens were selected from our field-sample. Relative molecular weights of serum albumins were determined by comparison with the distance traveled by marker proteins. Patterns of migration for local and non-local *X. muelleri* and *X. laevis* were in agreement with previously published accounts, *X. muelleri* albumin proteins having an intense band of approximately

70kDa, and *X. laevis*, a minor band of 70 kDa and more intense band of 74kDa (Fischer *et al.*, 2000) (Figure 7-23). Also in agreement with earlier work was the additive expression, in F1 hybrids, of both bands (Graf and Fischberg, 1986) (Figure 7-23). This result matched that found by Fischer *et al.* (2000) for their wild caught specimen, reported to have been an F1 hybrid. We were fortunate in being able to obtain a serum sample from an F2 backcross individual, which showed the same pattern of protein migration as the F1 hybrid. Serum samples from all specimens with intermediate morphology, and specimens from Pretoriuskop (intermediate mate calls) were processed. This sample included specimens whose HI scores ranged from 5-9. The pattern shown by each of these was identical, matching that of *X. laevis* (a selection of protein migration lanes is shown in Figure 7-23).

Figure 7-23

Scan of SDS-gel showing migration pattern of albumin proteins in *X. l. laevis*, *X. muelleri*, an intermediate and in an F1 hybrid, as representative selection of results gained from a larger sample.



Ploidy

Introduction

Cell volume is reported to correlate closely with nuclear DNA content (Manfredi and Romanini, 1973) and can be applied to determine relative ploidy (Schroer and Greven, 1998) in groups such as *Xenopus*. Whilst this has not yet been done, Reumer and Thiebaud (1987) correlated osteocyte diameter with ploidy in *Xenopus* whilst Kobel *et al.* (1996) correlated erythrocyte nucleus size with chromosome number in *Xenopus*.

A more precise evaluation of relative nuclear DNA volume in erythrocytes of different taxa can be obtained using flow-cytometry, a method previously shown by Thiebaud and Fischberg (1977), and by Fischer *et al.*, (2000) as being able to detect inter-species differences in relative DNA volume in *Xenopus*.

Materials and methods

The mean diameter of 25 erythrocyte nuclei was measured for each of 15 specimens of intermediate morphology, returned live from Southern Africa to the laboratory (max. and min. diameters of oval nuclei were divided by 2). Data for twelve additional specimens, six each of *X. muelleri* and *X. l. laevis* (local and non-local) were used for comparison with intermediates. Nuclei from a further two F1 hybrid backcrosses were also measured.

Blood was smeared over a microscope slide and allowed to dry prior to dehydration and fixation by immersion in methanol for 3 minutes and staining in dilute Giemsa (1:20 H_2O, pH 7) for 1 hour. Cells were differentiated in pH neutral buffer solution for 30 seconds, and examined light-microscopically at 100x using a Leica fitted with a calibrated eye-piece graticule.

Despite extensive modification of flow-cytometry methods used by Fischer *et al.*, (2000), this approach to determining relative ploidy in intermediates had to be abandoned owing to constraints imposed by time.

Results

Figure 7-24 demonstrates that mean nucleus diameter overlaps broadly between taxon groups, indicating that there is no variation in ploidy between backcross individuals, intermediates and either parent taxon, though actual erythrocyte diameter varies significantly only between F2 backcross individuals and intermediates (d.f.=3,25; F=4,10; p=0.017). Indications are that each taxon group is diploid with respect to *X. l. laevis* and *X. muelleri*.

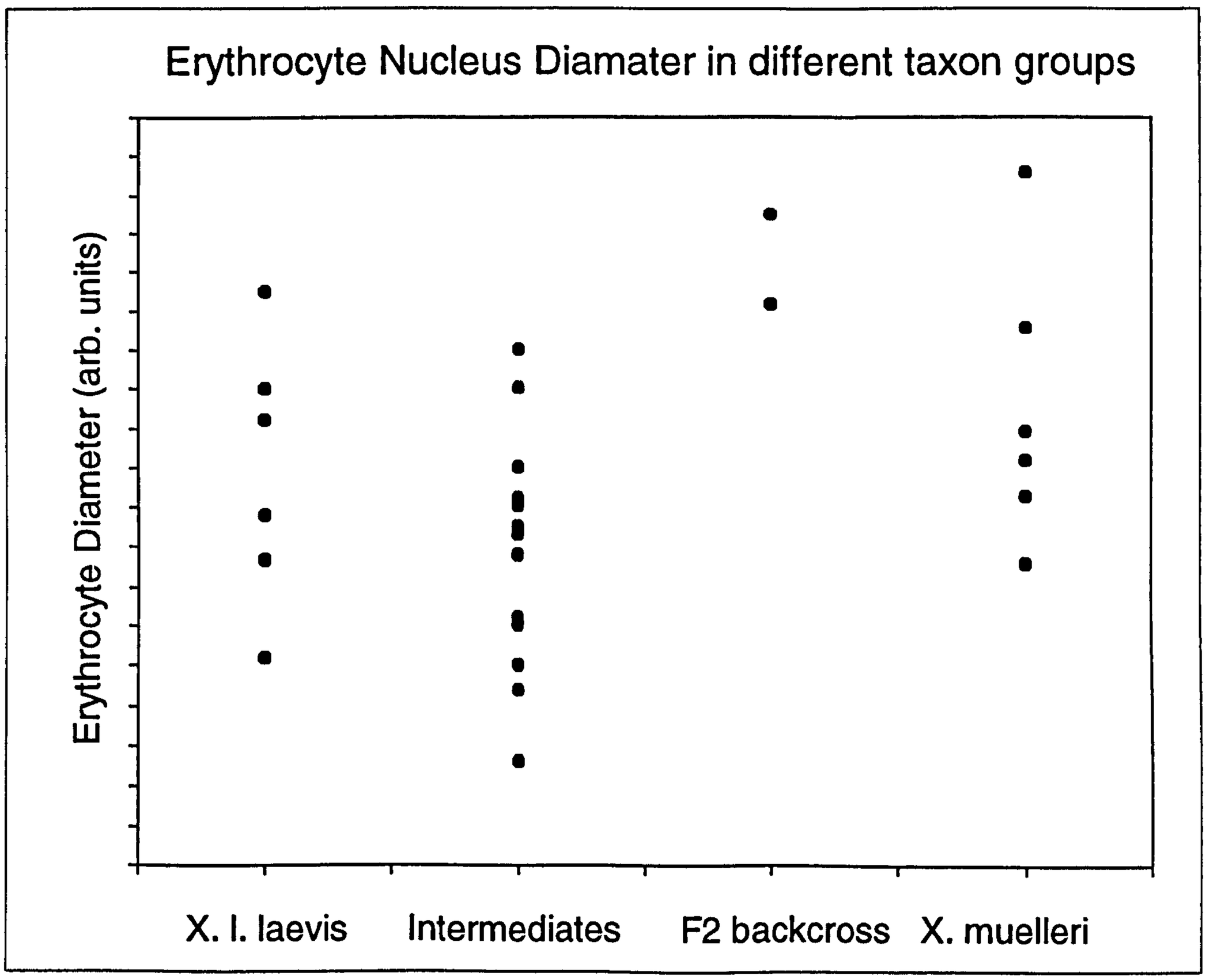


Figure 7-24

Indication of relative ploidy of intermediates, as compared with F2 backcross individuals and parent taxa. Data are of mean erythrocyte nucleus diameter of 25 cells per individual, and sample sizes are 6 for each pure taxon, 15 intermediates and 2 F2 backcross individuals.

Further Results

Joe Jackson has been continuing work on this material, and certain results obtained since I completed my period of lab-work are pertinent to further discussion. Specimens of intermediate morphology have successfully been crossed with each other and backcrossed with both *X. l. laevis* and *X. muelleri*- morphs from the area of study. Data for viability of eggs collected from crosses between intermediates collected in the field are low compared with pure species viability levels. In this respect, fertility appears to be compromised in these specimens.

The proportion of individuals infected with parasites is said to increase proportionally with introgression rate (LeBrun *et al.*, 1992). Prevalence of certain parasites (*Protopolystoma*) is remarkably high in a sample of intermediates collected from a mixed species site (Pretoriuskop). Further studies demonstrated that some wild-caught specimens of intermediate morphology harboured both of the two strictly host specific *Protopolystoma* species, suggesting that some intermediates may inherit dual susceptibility to infection from parent taxa. Dual infection has not been attempted for lab-reared hybrids and work on susceptibility to parasite infection in this system is ongoing.

DISCUSSION

Natural hybridization between *X. muelleri* and *X. l. laevis* appears to have been more widespread in nature than has been reported in the literature. Though F1 hybrids appear to be rare in our field-sample, evidence is presented of extensive introgression, a phenomenon supported by the relative ease with which specimens of intermediate morphology and F1 hybrids have been cross-bred in the laboratory.

As demonstrated by results from the present study, not all morphological intermediates were determined as such by other techniques. Likewise, many specimens determined by morphology to be pure species were demonstrated by other methods to contain introgressed genes. Results from analysis of morphology and osteometric data however, were largely in agreement with those demonstrating an increased rate of osteological aberrations in identifying specimens of intermediate taxonomic status. Osteometry was the more sensitive indicator in this respect, and whilst results were based on only a small proportion of available material, the sensitivity of this method makes appropriate its application to remaining material from this period of fieldwork with a view to describing taxonomic differentiation across the zone of hybridization.

In contrast, gel electrophoresis, used to compare albumin protein mobility of morphological intermediates with that of parent species, showed all specimens processed for the current study to demonstrate either a *X. l. laevis* or *X. muelleri* pattern of band migration. Each of these was consistent with the weighting of that individual's HI score, be it more *X. l. laevis* or *X. muelleri*-like. Joe Jackson has been continuing comparative work on albumin proteins and has demonstrated evidence of dual inheritance for this gene in a single specimen of intermediate morphology. This result matched those from the current study for lab-reared F1 hybrids and an F1 hybrid–*X. l. laevis* backcross, suggesting a hybrid or backcross status for this specimen. Joe Jackson has also examined expression of non-albumin blood proteins for this sample. Heterogeneity was demonstrated for a single *X. muelleri*-morph, providing strong evidence of extensive introgression in the direction of this taxon. This parallels a similar result obtained by John Measey (pers. com.) who demonstrated the expression of *X. l. laevis* mtDNA in a *X. muelleri*-morph from this field sample. Clearly, the effects of hybridization extend further than is suggested by morphology alone.

Features of the mate-call are believed to be under polygenic control (Mousseau and Howard, 1998), and are perhaps more predisposed to expressing evidence of dual inheritance than either albumin protein or mtDNA. As such, inter-species comparison of mate-call in a hybrid zone provides the potential for a highly sensitive means of determining the taxonomic status of specimens. Field observations have revealed evidence that the breeding seasons of both species coincide temporally. This evidence, combined with reports that the mating-call is a poor pre-mating inter-species barrier,

would predict a high rate of miss-pairings in this hybrid zone. Results from the present study support this prediction in having shown widespread evidence of hybridization between *X. muelleri* and *X. l. laevis*. Analysis of mate-call in specimens of intermediate morphology unequivocally demonstrated that the calls of all morphologically intermediate males from a single, mixed-species site were intermediate between those of pure parent species. Furthermore, this study has demonstrated that even the calls of males chosen on the basis of morphology to represent pure *X. l. laevis* were intermediate with respect to those of non-local *X. l. laevis* collected from an allopatric site.

The graded sensitivity and relatively unobtrusive nature of this approach make it an invaluable methodological tool, appropriate not only for laboratory studies but also for application in the field. Indeed mate-call, more specifically inter-click interval, has been demonstrated to be useful for species identification in a number of anuran groups: bufonids (Sullivan and Leek, 1987; Brauer, 1991), *Hyla* (Littlejohn, Fouquette and Johnson, 1960; Bogart, 1980), leptodactylids (Martino and Sinsch, 2002) and *Neobatrachus* (Stock 1998). There is potential for digital characterization of the calls of *X. muelleri* and *X. l. laevis* for use in identifying hybrids in the field using an artificial neural network (Stuart Parsons, *pers. com.*). This would enable the mapping of areas of hybridization without the need for extensive collecting and invasive techniques.

Non-congruence

Non-congruence of results between some of the techniques used to determine taxonomic status is probably the result of uncoupling of genes controlling for these characters during the process of introgression. In such a situation, a differential would develop in the response of different genes to selection forces applied by a range of subtle ecological gradients. This process would result, over time, in disparity in the pattern of introgression expressed by different characters throughout the population (Gollmann, 1991). It is generally believed that the greater the differential between characters, the more historic the hybrid zone (Klingenberg *et al.*, 2000). Every indication from results of the current study suggests that the hybrid zone between *X. muelleri* and *X. l. laevis* is indeed historic. If this were the case, it would be expected that hybrids should occur in museum collections from the area (Picker, 1985), providing great potential in terms of determining the range, age and stability of this zone.

Biogeographic explanation for contact zone

This study area falls within an ecological transition zone that is characterized as separating the amphibian faunas of Afromontane and lowland habitats (Poynton and Boycott, 1996). It is further recognized that over a quarter of hybrid zones are found near such areas of ecological transition and are the result of secondary contact following the last glacial maxima (Littlejohn, 1973; Hewitt and Mitchell, 1998). Poynton and Boycott (1996) suggested that the ranges of these two species will have undergone reciprocal expansion and contraction during the course of cyclical climatic changes of the

Pleistocene, a theory supported by distribution data which demonstrated isolated populations of *X. l. laevis* occupying mountain islands in the lowveld (Poynton and Boycott, 1996). A likely explanation for such a disjunct pattern would be that *X. l. laevis* formally occupied a wider area, probably during cooler, more arid periods, and left relict island populations in the lowveld following subsequent range contraction to higher, inland areas during interglacial periods. Although not yet documented, it is possible that lowland island pockets of *X. muelleri* exist within an area otherwise occupied by *X. l. laevis*, relict distributions from past, warmer periods when the distribution range of *X. muelleri* was more extensive.

Given the apparent extent of hybridization and introgression in the contact area that forms the focal point of the present study, it is likely that hybridization, followed by introgression, occurs wherever these two species come into contact both along the main zone of parapatry along the foot of the Drakensburg Escarpment and also at the periphery of lowveld island population ranges of *X. l. laevis*. The same could be true of inland *X. muelleri* populations, were they to exist. Assuming from evidence of non-congruence between results from different methodological approaches that hybridization is not a recent event in the history of contact between their respective ranges, one could assume that hybridization followed by introgression will have occurred during each successive period of contact between the ranges of parent taxa throughout the Pleistocene.

That introgression proceeds from hybridization and is extensive across the study area brings into consideration the possibility that genetic introgression may pose a threat to the integrity of parent taxa. Propagation of 'non-self' genes through either population by extensive introgression would explain the expression of non-self characters in pure species morphs. The rate of spread of 'non-self' genes through a population would be proportional to the rate of hybridization and subsequent survival and fecundity of backcross individuals, the rate of hybridization itself being a function of the geographic extent of a contact zone. The discovery of *X. l. laevis* island populations within otherwise *X. muelleri* habitats (Poynton and Boycott, 1996) suggests that the rate of introgression of *X. l. laevis* genes into the *X. muelleri* population would be greater than if the only geographical interface between these taxa were along one main contact zone. The very great geographic extent of distribution ranges of both *X. l. laevis* and *X. muelleri* is such however, that whilst possible, it is unlikely that hybridization along contact zones will pose a serious threat to the genetic integrity of either species. Even if the effects of introgression were geographically extensive (as is indicated by results from chapter 6), gene transfer between species need not be disadvantageous, and need not compromise the genetic fitness of either population (Fischer *et al.*, 2000). Indeed it may actually confer certain advantages upon the recipient population, extending its genetic repertoire to encompass greater adaptive plasticity. This would have obvious advantages for species living in an environment that has

been demonstrated to have undergone extreme habitat disturbance in the past. Compromised fertility may be incurred as an inevitable cost however.

CONCLUSION

Survival in marginal habitats is known to promote opportunism and resilience (Fjeldsa, Ehrlich, Lambin and Pins, 1997). It is probable that the ranges of both *X. l. laevis* and *X. muelleri* will have undergone episodes of considerable habitat disturbance during Pleistocene periods of climatic flux, placing on these taxa a high degree of survival pressure. This has probably contributed towards these species acquiring a wide ecological tolerance (Picker *et al.*, 1996), extensive distribution range and generalist lifestyle (Tinsley *et al.*, 1996). It is possible that the ability to hybridize is also linked with their environmental history, and has functioned to increase their ability to survive in a dynamic landscape.

Results of extensive hybridization and introgression from the present study suggest that hybridization has occurred whenever the ranges of these two species have come into contact, an event that will have occurred repeatedly throughout the Pleistocene, and continues today. Whether non-self genes are propagated throughout populations by extensive introgression or are contained within the immediate zone of hybridization (*sensu* Short, 1969; Littlejohn, 1969) will depend on a number of ecological factors. Given the apparently historic nature of hybridization between these two taxa, examination of museum collections and further fieldwork both promise great potential in terms of gaining a more thorough understanding of the dynamics of this interaction.

BIBLIOGRAPHY

Acocks, J.P.H. (1975). Veld types of South Africa. *Memoirs of the botanical survey of South Africa* **40**:1-128.

Barton, N.H. and Hewitt, G.M. (1989). Adaptation, speciation and hybrid zones. *Nature* **341**:497-502.

Bellis, E.D. (1957). The effects of temperature on salientian breeding calls. *Copeia* **1957**: 85-89.

Bisbee, Ch.A., Baker, M.A., Wilson, A.C., Hadji-Azimi, I. and Fischberg, M. (1977). Albumin phylogeny for clawed frogs (*Xenopus*). *Science* **195**:785-787.

Bogart, J.P. 1980. "Evolutionary implications of polyploidy in amphibians and reptiles." Pp. 341-377 in Polyploidy: Biological Relevance. 1st ed., edited by Lewis, W.H. London: Plenum Press.

Brauer, K. 1991. Kröten. Leipzig, C. Jena, Berlin, Urania.

Le Brun, N., Renaud, F., Berrebi, P. and Lambert, A. (1992). Hybrid zones and host parasite relationships: Effect on the evolution of parasitic infection. *Evolution* **46 (1)**:56-61.

Corti, M. and Thorpe, R.S. (1989). Morphological clines across a karyotypic zone of house mice in Central Italy. *Journal of Evoloutionary Biology* **2**:253-264.

Cooke, H.B.S. (1962). The Pleistocene environment in southern Africa during the Pleistocene. *Annals of the Cape Province Museum of Natural History* **2**:11-15.

Diamond, A.W. and Hamilton, A.C. (1980). The distribution of forest passerine birds and quaternary climatic change in Tropical Africa. *Journal of Zoology, London* **191**:379-402.

Evans, B.J., Morales, J.C., Picker, D.J., Melnick, D.J. and Kelley, D.C. (1998). Absence of extensive introgression between *Xenopus gilli* and *Xenopus laevis laevis* (Anura: Pipidae) in Southwestern Cape Province, South Africa. *Copeia* **1998**:504-509.

Fischer, W.J., Koch, W.A. and Elepfandt, A. (2000). Sympatry and hybridization between the clawed frogs *Xenopus laevis laevis* and *Xenopus muelleri* (Pipidae). *Journal of Zoology, London* **252**:99-107.

Fjeldsa, J., Ehrlich, D., Lambin, E. and Pins, E. (1997). Are biodiversity 'Hotspots' correlated with current ecoclimatic stability? A pilot Study Using the NOAA-AVHRR Remote Sensing Data. *Biodiversity and Conservation* **6**:401-422.

Gollmann, G. (1991). Osteological variation in *Geocrinia laevis*, *Geocrinia victoriana*, and their hybrid populations (Amphibia, Anura, Myobatrachinae). *Zeitschrift fuer zollogische systematic und evolutionsforschung* **29**:289-303.

Gollmann, G., Roth, P. and Hodl, W. (1988). Hybridization between the fire-bellied toads *Bombina bombina* and *Bombina variegata* in the Karst regions of Slovakia and Hungary: morphological and allozyme evidence. *Journal of evolutionary biology* **1**:3-14.

Graf, J.-D. and Fischberg, M. (1986). Albumin evolution in polyploid species of the genus *Xenopus*. *Biochemical Genetics* **24**: 821-837.

Graf, J.-D. 1996. "Molecular approaches to the phylogeny of *Xenopus*". Pp.379-388 in The biology of *Xenopus*, edited by Tinsley, R.C., and Kobel, H.R. Oxford: Clarendon Press.

Hewitt, G.M. (1988). Hybrid zones- natural laboratories for evolutionary studies. *Trends in Ecology and Evolution (TREE)* **3**:158-166.

Hewitt, G.M. and Mitchell, J.F.B. (1998). A fully coupled GCM simulation of the climate of the mid-Holocene. *Geophysical Research letters* **25**:361-364.

Klingenberg, C.P., Spence, J.R. and Mirth, C.K. (2000). Introgressive hybridization between two species of waterstriders (Hemiptera: Gerridae: *Limnoporus*): geographical structure and temporal change of a hybrid zone. *Journal of evolutionary biology* **13**:756-765.

Kobel, H.R., Loumont, C. and Tinsley, R.C. (1996). "The extant species." Pp. 9-33 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Kobel, H.R., Barandun, B. and Thiébaud, ChH. (1998). Mitochondrial rDNA phylogeny in *Xenopus*. *Herpetological Journal* **8 (1)**:13-18.

Kobel, H.R., Du Pasquier, L. and Tinsley, R.C. (1981). Natural hybridization and gene introgression between *Xenopus gilli* and *Xenopus laevis laevis* (Anura: Pipidae). *Journal of Zoology, London* **194**:317-322.

Lawes, K.J. (1990). The distribution of the Samango monkey (*Cercopithecus mitis erythrarchus* Peters, 1852 and *Cercopithecus mitis labiatus* I. Geoffroy, 1843) and forest history in southern Africa. *Journal of Biogeography* **17**:669-680.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**:680-685.

Littlejohn, M.J. (1969). "The systematic significance of isolating mechanisms." In Systematic biology, Proceedings of an International Conference. National Academy of Science: Washington.

Lambiris, A.J.L. (1988). A review of the amphibians of Natal. *Lammergeyer* **39**:1-191.

Littlejohn, M.J., Fouquette, M.J. and Johnson,C. (1960). Call discrimination by female frogs of the *Hyla versicolor*-complex. *Copeia* **1960**:47-49.

Littlejohn, M.J., Watson, G.F. and Loftus-Hills, J.J. (1971). Contact hybridization in the *Crinia laevis* complex (Anura: Leptodactylidae). *Australian Journal of Zoology* **19**:85-100.

Littlejohn, M.J. and Watson, G.F. (1973). Mating-call variation across a narrow hybrid zone between *Crinia laevis* and *C. victoriana* (Anura: Leptodactylidae). *Australian Journal of Zoology* **21**:277-284.

Manfredi Romanini, M.G. 1973. "The DNA nuclear content and the evolution of vertebrates" Pp. 39-81 in Cytotaxonomy and vertebrate evolution. Chiarelli, A.B., Capanna, E. eds., London, New York, Academic Press.

Martino, A.L. and Sinsch, U. (2002). Speciation by polyploidy in *Odontophrynus americanus*. *Journal of Zoology, London* **257**:67-81.

Moore, W.S. and Buchanan, D.B. (1985). Stability of the northern Flicker hybrid zone in historical times: implications for adaptive speciation theory. *Evolution* **39(1)**:135-151.

Muller, W.P. (1966). Diplotene chromosomes of *Xenopus* hybrid oocytes. *Chromosoma* **59**:273-282.

Musseau, T.A. and Howard, D.J. (1998). Genetic variation in cricket calling song across a hybrid zone between two sibling species. *Evolution* **52(4)**:1104-1110.

Olson, D.C., Dinerstein, E., Wikramanayake, E.D., Burgess, N.D., Powell, G.V.N., Underwood, E., D'Amico, J.A., Itoua, I.I., Strand, H.E., Morrison, J.C., Loucks, C.J., Allnutt, T.F., Lamoreux, J.F., Ricketts, T.H., Kura, Y., Lamoreux, J.F., Wettengel, W.W., Hedao, P. and Kassem, K. (2001). Terrestrial ecoregions of the world: a new map of life on earth. *Bioscience* **51(11)**: 933-938.

Passmore, N.I. and Carruthers V.C. 1995. South African frogs. Johannesburg: Witwaterstrand University Press.

Picker, D. (1980). *Xenopus laevis* (Anura; Pipidae) mating systems- a preliminary synthesis with some data on the female phonoresponse. *South African Journal of Zoology* **15(3)**:150-158.

Picker, M.D. (1985). Hybridization and habitat selection in *Xenopus gilli* and *Xenopus laevis* in the south-western Cape Province. *Copeia* **1985**:574-580.

Picker, M.D., Harrison, J.A. and Wallace, D. (1996). "Natural hybridization between *X. l. laevis* and *X. gilli* in the south-western Cape Province, South Africa". Pp. 61-71 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Poynton, J.C. (1964). The Amphibia of South Africa: A faunal study. *Annals of Natal Museum* **17**:1-334.

Poynton, J.C. (1998). Tanzanian Bufonid diversity: preliminary findings. *Herpetological Journal* **8 (1)**:3-6.

Poynton, J.C. and Boycott, R (1996). Species turnover between Afromontane and east African lowland faunas. Patterns shown by amphibians. *Journal of Biogeography* **23**:669-680.

Poynton, J.C. and Broadley, D.G. (1985). Amphibia Zambesiaca 1. Scolecomorphidae, Pipidae, Microhylidae, Hemisidae. Arthroleptidae. *Annals of Natal Museum* **26**:503-553.

Rau, R.E. (1978). The development of *Xenopus gilli* Rose and Hewitt (Anura, Pipidae). *Annals of the South African Museum* **76**:247-263.

Reumer, J.W.F. and Thiebauld, C.H. (1987). Osteocyte lacunae size in the genus *Xenopus* (Pipidae). *Amphibia-Reptilia* **8**:315-320.

Schroer, T. and Greven, H. (1998). Verbreitung, Populationsstrukturen und ploidiegrade von Wasserfroschen in westfalen. *Zeitschrift feldeherpetol.* **5**:1-15.

Scriven, P.N. and Bachau, V. (1992). The effect of hybridization on mandible morphology in an island population of the house mouse. *Journal of Zoology, London* **226**:573-583.

Short, L.L. (1969). Taxonomic aspect of avian hybridization. *Auk* **86**:84-105.

Stock, M. (1998). Mating call differences between diploid and tetraploid green toads (*Bufo viridis* complex) in Middle Asia. *Amphibia-Reptila* **19**:29-42.

Stuckenberg, B.R. (1969). Effective temperature as an ecological factor in southern Africa. *Zoologica Africana* 4:145-197.

Sullivan, B.K. (1995). Temporal stability in hybridization between *Bufo microscaphus* and *Bufo woodhousii* (Anura: Bufonidae): Behavior and morphology. *Journal of evolutionary biology* 8:233-247.

Sullivan, B.K. and Lamb, T. (1988). Hybridization between the toads *Bufo microscaphus* and *Bufo woodhousei* in Arizona: variation in release call and allozymes. *Herpetologica* 44:325-333.

Sullivan, B.K. and Leek, C. (1997). Acoustic communication in Woodhouse's toad (*Bufo woodhousei*). II. Response of females to variation in spectral and temporal components of advertisment calls. *Behaviour* 103:16-26.

Thiebaud, C.H., and Fischberg, M. (1977). DNA content in the genus *Xenopus*. *Chromosoma* 59:253-257.

Thorpe, R.S. (1988). Multiple group Principal Component Analysis and population differentiation. *Journal of Zoology, London* 216:37-40.

Tinsley, R.C. and Kobel, H.R., eds. 1996. The Biology of *Xenopus* 1st ed. Oxford: Clarendon Press.

Tinsley, R.C., Loumont, C. and Kobel, H.R. 1996. "Geographical distribution and ecology". Pp. 35-59 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Vigny, C. (1979). The mating calls of 12 species and sub-species of the genus *Xenopus* (Amphibia: Anura). *Journal of Zoology, London* 188:103-122.

van Zinderen Bakker, R. ed. 1972. Palaeoecology of Africa 1st ed., Vol. 9. Cape Town/ Rotterdam: A.A. Balkema.

van Zinderen Bakker, R. (1978). "Quaternary vegetation changes in Southern Africa." In Biogeography and Ecology of Southern Africa. Monographie and Biologicae 3i. Ed. by M.J.A.Werger. Junk. The Hague.

Westley, B., Wyler, T., Ryffel. G. and Weber, R. (1981). *Xenopus laevis* serum albumins are encoded in two closely related genes. *Nucleic acids Research* 9:3557-3574.

Yager, D.D. 1996. "Sound production and acoustic communication in *Xenopus borealis*." Pp. 121-141 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

PAGE

NUMBERING

AS ORIGINAL

CHAPTER 8 : OSTEOLOGY OF *XENOPUS LARGENI* FROM THE HIGHLANDS OF ETHIOPIA AND INTERPRETATION OF EVOLUTIONARY RELATIONSHIPS WITH OTHER PIPIDAE.

INTRODUCTION

For nearly a century, *Xenopus clivii* Peracca, 1898 was the only *Xenopus* species recorded from Ethiopia (Kobel, Loumont, Tinsley, 1996). A second species, *X. largeni* Tinsley, 1995 from the Bale Mountains of Ethiopia first came to light as Tinsley examined museum material labeled *X. clivii* (*see* Tinsley, 1995). A subsequent field trip to the *X. largeni* locality, as identified by the museum record, provided live material that formed the basis of the 1995 description (Tinsley, 1995).

The same material was used in a number of studies that attempted to elucidate the systematic position of the species. Protein electrophoresis, karyotype, mating-call, biochemistry and genetics each allied *X. largeni* more closely to *X. laevis* than any other species (Mann, Risley, Eckhardt and Kasinsky, 1982; Burki, Schwager and Fischberg, 1984; Burki and Fischberg, 1985; Graf and Fischberg, 1986; Kobel, 1996; Tymowska, 1991; Loumont [published in Tinsley, 1995]). The same studies showed the second Ethiopian species, *X. clivii*, to be most closely related to its nearest geographic neighbours, *X. muelleri* and *X. borealis*. Recent studies however, have shown both *X. clivii* and *X. largeni* to occupy a relatively isolated position, systematically, with respect to other *Xenopus* species (Kobel, Barandun and Thiebaud, 1998).

Biogeography

Both Ethiopian species are believed to be endemic to these highlands, extending also into Eritrea in the case of *X. clivii* (see Tinsley, 1995). *Xenopus clivii* is widespread at a range of altitudes exceeding 1500m, whilst *X. largeni* is known only from a small number of sites between 1500 and 2650m, and is known to be sympatric with the former species in one site at 2600m in the Bale mountains region of Ethiopia.

That the only two *Xenopus* species known from Ethiopia are endemic to these highlands is consistent with the unusual biogeographic pattern that characterizes the country. The importance of the Ethiopian highlands as a center of endemism has been confirmed by several distribution studies carried out on a range of groups, including flora, mammals, avifauna, amphibians and snails (Yalden, Largen, Hock and Hillmann, 1996; Bidgood and Friis, 1998; Largen, 1998; Largen, 2001).

In a recent review of the herpetofauna of Ethiopia, almost half of the species there were found to be endemic (Largen, 1998, 2001). Eighty percent of these endemics occur above an altitude

threshold of 1800m in areas of montane grass-, moor- and woodland (Largen, 1998; WWF 1998), and are concentrated in the Bale Mountains of southern Ethiopia, the area occupied by *X. largeni.*

Somewhat paradoxically, for a country postulated to have contained an ice-age refugium, Ethiopia is also characterized by an impoverished fauna when compared with corresponding refuge areas in East Africa, Southwestern Uganda, the Eastern Arc Mountains and Cameroon (Carcasson, 1964; Werger, 1978; Hamilton, 1982; Yalden *et al.*, 1996; Fjeldsa, 1997; Fjeldsa and Lovett, 1997; Brown, 2001).

The key to understanding this unusual biogeographic pattern lies in the geography of the country and its effects on recent climatic history. The primary geographic factor that distinguishes Ethiopia from other postulated highland refugia is the sheer extent of high altitude land; remarkably, 80% of land over 3000m, and 50% of land over 2000m south of the tropic of Cancer, lies in Ethiopia (Largen, 1998). This accounts in part for the long-standing history of aridity that affects Ethiopia (Hamilton, 1982). A 'climatic catastrophe - probably an unmitigating dry season' has been proposed as the only possible explanation for the observed degree of species poverty in these highlands (Hamilton, 1976).

Of equal significance is its separation from areas to the south by the formidable *Acacia-Commiphora* scrub-desert of the Northern Frontier district of Kenya and Eastern Sudan (Carcasson *et al.*, 1964; WWF ecoregion map, 1998). Contact between forests of Ethiopia and those to the south would call for a considerable increase in precipitation in these intervening lowlands and a greater depression of forest belts than has been calculated for inter-glacials of the Pleistocene (Hedberg, 1969). As such, a steep gradient of habitat differentiation between the Ethiopian highlands and surrounding lowland areas is postulated to have prevailed throughout much, if not all of the Pleistocene, and will have presented a long-term barrier to the migration of taxa to and from neighbouring montane forests, accounting for the high levels of endemism currently associated with these highlands (Carcasson *et al.*, 1964; Hedberg, 1969; Bidgood and Friis, 1998; DeKlerk, 2002).

AIM

The original aim of this study was to assess osteological evidence for the determination of relationships between Ethiopian species and other species within the genus. Collections of *X. clivii* are very rare in European and American museums and whilst permission was denied for osteological preparation of those few specimens, permission to x-ray was granted. A small number of *X. largeni* specimens was acquired for osteological preparation however, and additional specimens borrowed for the purpose of x-ray. These have formed the basis of the current comparative osteological study.

Only a small number of references to the osteology of *X. largeni* exist in the literature. One is presented in the form of illustrations of vertebral column and pelvic girdle in a chapter on the general

osteology of pipids (Trueb, 1996). However no specific reference to this species is made in the text. The others refer only to a single element. The vomer is said to be paired (Baez and Rage, 1998; Baez and Trueb, 1997), and the atlas neural arch indented (Baez and Pugener, 1998). A full descriptive account of the osteology of *X. largeni* will therefore also form part of the study, along with a list of the main features that distinguish the taxon from other *Xenopus* species examined during the course of this study. The evolutionary significance of some of these features will be assessed.

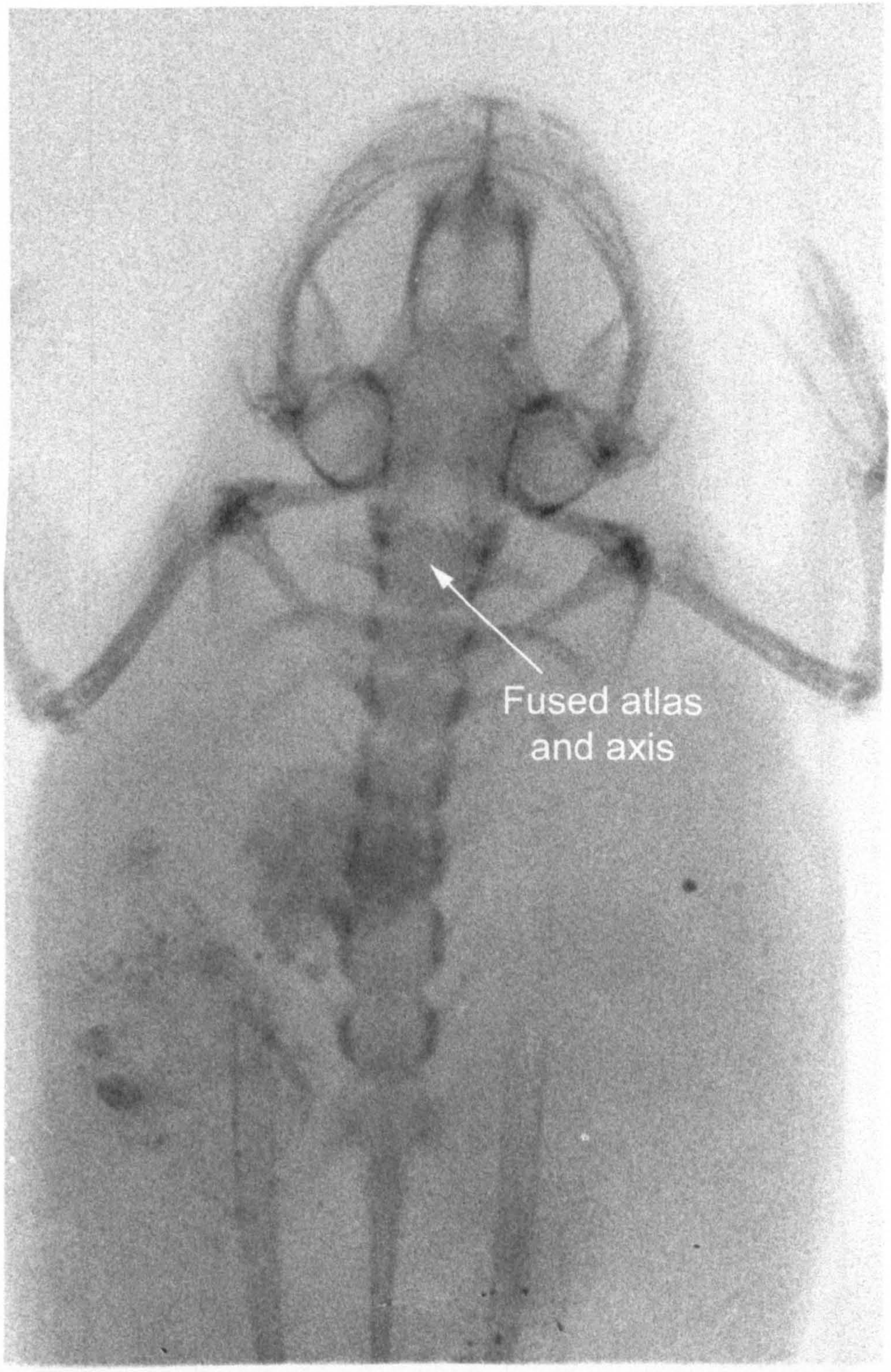
MATERIALS AND METHODS

Material was loaned for X-ray by the AMNH and BMNH. Material was acquired for dissection from R.C. Tinsley. Three *X. largeni* specimens from the AMNH (A-158387, A-158388, A-158389), and 3 specimens each of *X. largeni* and *X. clivii* from the BMNH were X-rayed (BM1977.873, BM1974.2497-2498; BM1977.867, BM1976.2021-2022). Taxa used for inter-species comparisons are listed in materials sections throughout the thesis. X-rays were taken at the pre- and post-clinical veterinary school, University of Bristol and at the Long Ashton dental surgery, Bristol. Those from the dental practice gave the clearest images, and were taken using a Phillips Denso-mat, set to patient type N, max. 4-5 premolars. *Xenopus largeni* from R.C. Tinsley's private collection were prepared for osteological examination. Seven specimens were used in the comparative study, 5 males (L1, L2, L3, L5, L7) and 2 females (L4, L6). These were siblings reared in the laboratory from two out of three available specimens collected from Sidamo Province, Ethiopia. Each was prepared, examined and measured following methods outlined in chapter 3.

RESULTS

Despite repeated attempts at x-ray using facilities at the pre-clinical and clinical vet school, University of Bristol, and the Long Ashton dental surgery, Bristol, no image was sufficiently sharp to be of use in characterizing either *X. clivii* or *X. largeni* in any degree of detail. It was possible however, to determine whether or not atlas and axis were fused. In *X. largeni*, these elements were fully fused in all specimens examined, whilst in *X. clivii*, elements remained separate. All remaining results are based on skeletal preparations of *X. largeni*, provided by R. C. Tinsley. The osteology of *Xenopus clivii* remains to be described (see Figure 8-1, Figure 8-2).

Mean snout-vent length (svl) for males was 40.76mm (±4.5 s.d.), and for females 37.5mm (±3.57s.d.); for the sample as a whole 39.84 (±4.25s.d.). This direction of discrepancy in s-v length between sexes is opposite to that which would be expected. However specimens were considered to be sexually mature, since the ovaries of each female specimen contained a small number of mature

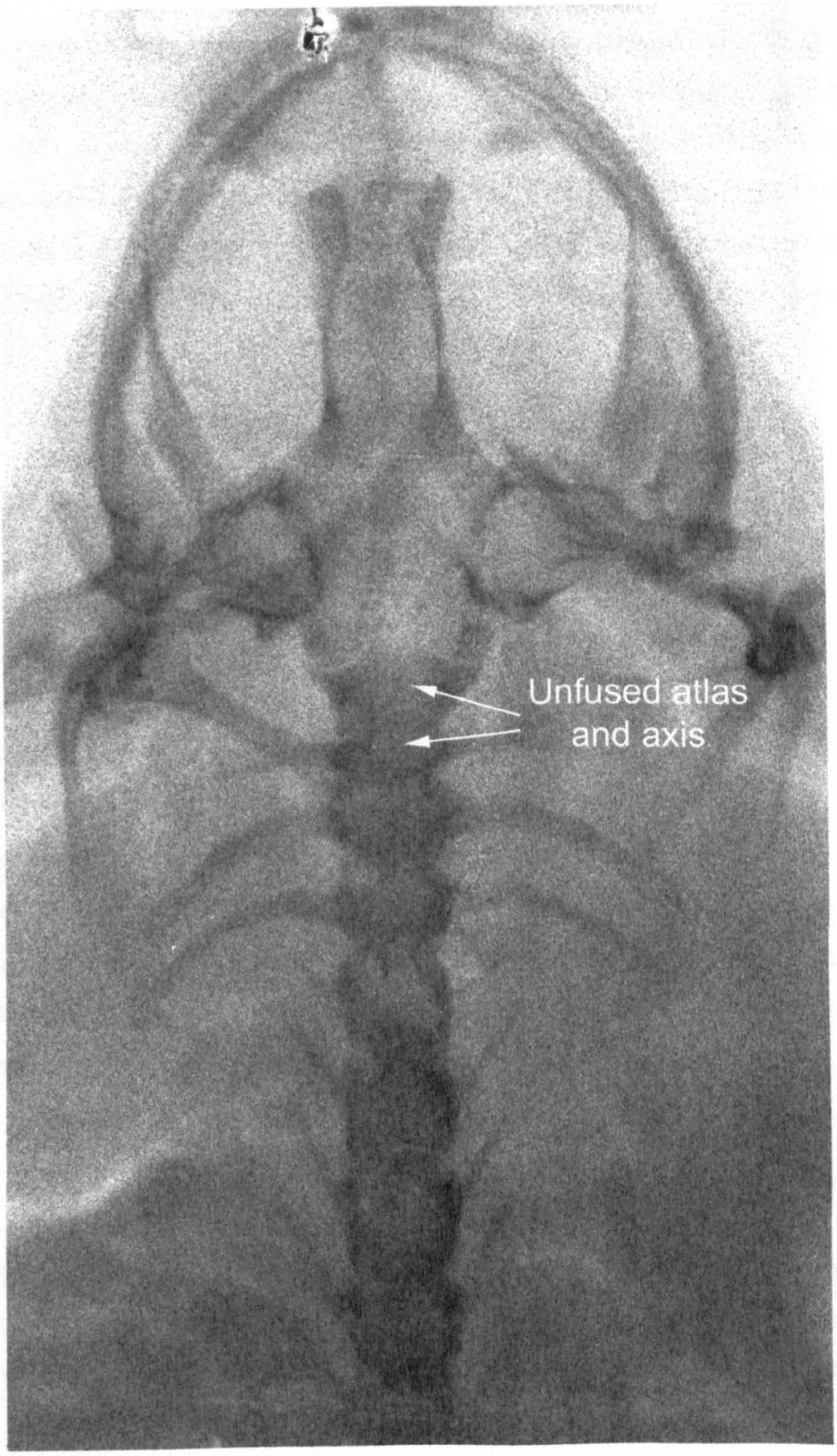


ova, and although males lacked gloves, testes were large in each specimen and the larynx was highly ossified.

Scale: _____ 1mm

Figure 8-1

X-ray of *X. largeni* showing fused atlas and axis.



Scale: _____ 1mm

Figure 8-2

X-ray of *X. clivii* showing unfused atlas and axis. Scale: x5

Description of the osteology of *X. largeni*

<u>*Cranium*</u>

Frontoparietal

The frontoparietal is extremely broad (Illustration 8-1). Anterior margins are oriented steeply with respect to one another, forming an acute or squarely-angled apex. The element is waisted, widening anteriorly towards moderately well-developed anterolateral alae and posteriorly towards its lateral site of association with the occipitopetrosal. The caudal profile varies from being square-shaped to being evenly rounded, and lacks the distinct alae characteristic of other examined taxa. Parasagittal crests border a skull table that measures approximately a third the width of the frontoparietal in dorsal view. Crests are parallel along most of their length, diverging to meet anterolateral corners of the element, whilst converging to form a broad U-shaped posterior margin, well within the caudal margin of the element, delimiting a parabolic skull table. The medially positioned pineal foramen occurs variably between the level of anterolateral alae and approximately one-third of the way along the length of the element. The element is moderately flat in transverse section and in all cases is fused to the sphenethmoid laterally.

Sphenethmoid, vomer (and palatine)

This element is distinct in several respects (Illustration 8-2). The sphenethmoid is wide and bears an unusually broad, tapered cultriform process, which extends as far as the dental ridge of pre-maxillaries. Flanking the broad base of this rostral process are small, paired, widely separated, triangular, vomerine elements. These are edentate. The exception to this is L5, in which each vomerine element is paired. An additional pair of dermal elements is present in a second specimen, L7; these are positioned anterolateral to vomers, and are free from association with any other elements and the planum antorbitale. Orbitonasal foramina are entirely enclosed in bone within the anterolateral margin of the element. Optic foramina, again enclosed in bone, are very small and are positioned within the margins of retractor bulbi muscle scars (rtbs), opening laterally. Paired rtbs are pronounced and widely spaced in all but one specimen (L4), in which they are in medial contact. Posterolaterally, the element widens to articulate intimately with otic plates of the pterygoid by means of posterolateral alae, before narrowing to form a bulbous, spatulate, caudal process (the parasphenoid), extending almost to the level of the foramen magnum.

Occipitopetrosal

These paired elements are fused medially around the foramen magnum, narrowing laterally, where moderately well-developed crista parotica partially overlap compound tympanosquamosals (Illustration 8-1, Illustration 8-2). Occipitopetrosals articulate closely with the frontoparietal dorsally

and sphenethmoid ventrally. They form a posterior border to prootic foramina, which in turn are bordered anterodorsally by the frontoparietal, and anteroventrally by the sphenethmoid.

Anterior margins of the element are oriented perpendicular to the main axis of the body, whilst posterior margins are oriented obliquely, the element narrowing laterally towards the crista parotica. Dorsally, a pair of diagonal ridges extends posterolaterally from the site of association with the frontoparietal as far as the posterolateral margin of occipitopetrosals. Ridges have a short, posteromedial branch, which diverges at right angles from the main ridge, extending towards the foramen magnum. Lateral to these ridges, the element is gently convex in profile.

Ventrally, the element bears a pair of deep Eustachian troughs. These curve anterolaterally, circumventing auditory capsules, and are bordered posteriorly by a ridge that provides a site of attachment for ligaments of the otic plates. Posterior to this ridge, the element assumes a convex profile, interrupted only by a pair of ventral prominences that provide a site of anchorage for cervical muscles. Posteroventrally, the foramen magnum is bordered by a pair of deep jugular foramina, divided by an internal septum into medial jugular and lateral internal perilymphatic foramen.

Maxillary and pre-maxillary

The upper jaw is robust and is formed from paired pre-maxillaries and maxillaries (Illustration 8-2). These are supported by the cartilaginous planum antorbitale anteromedially, which is synchondrotically fused to the sphenethmoid anterolaterally. Support for the maxillary arcade is also provided posteriorly by the pterygoid. Paired maxillary and pre-maxillary elements associate by means of an oblique, caudally-overlapping junction. The maxillary arch extends between one third and half the length of the orbit, and is square-ended or broadly tapered distally. The arch reaches just short of the posterior margin of the anterior pterygoid ramus.

Pre-maxillaries bear deep palatine ridges; concave posterior margins accentuate the length of posteromedial and lateral margins. These paired elements have a robust anteromedial margin and bear dorsally projecting alary processes (not visible in ventral view).

Maxillaries are tri-radiate in cross section, bearing dental rows ventrolaterally, and facial and palatine ridges which are laterally and medially directed respectively. Teeth are non-pedicellate and monocuspid.

The dental row extends along the length of pre-maxillaries and just over half the length of maxillaries. Teeth are very small and are arranged in a disordered manner along the ridge, averaging 14 for pre-maxillaries and 26 for maxillaries, the total number of teeth averaging 40.

The pars facialis of maxillaries is short, extending only along the portions of maxillaries which are anterior to the orbit, diminishing beyond this point posteriorly, whilst anteriorly, the pars facialis extends beyond the anterior margin of maxillaries towards alary processes, providing

mechanical protection for nasal cavities. The pars palatina is widest at the junction separating pre-maxillaries, narrowing along the length of maxillaries.

Pterygoid

The pterygoid forms part of the lateral suspensorium that supports the maxillary arch (Illustration 8-1, Illustration 8-2). The element is tri-radiate: a long anteriorly-directed component (anterior ramus) articulates with the dorsal surface of the maxillary by means of a moveable articulation (and will be divided into [distal] anterior ramus and [proximal] ventromedial flange for ease of description), a medially directed otic plate (medial ramus) that forms a floor to the Eustachian canal and rests on the pterygoid knob medially, and a laterally directed component (posterior ramus) that associates with the pars articularis.

The anterior ramus is dorsomedially directed with minimal anterolateral curvature. The ventromedially directed flange is more robust and deeper in transverse profile, bearing a small notch along its dorsal margin. The medial ramus of the pterygoid is relatively broad and short and serves as a floor to the Eustachian trough. This ramus, which is not bifid, forms a broad spatulate process, which obscures the pseudobasal process/ pterygoid knob and extends medially.

Tympanosquamosal and columella

This tri-radiate element is the product of fusion between tympanic annulus and squamosal and forms the lateral-most part of the skull (Illustration 8-1) The tympanic annulus is a conch-shaped element that houses the columella or stapes. A long narrow element that is anchored at its broad pars internal plectri to the fenestra ovalis of the occipitopetrosal, extending anterolaterally through a furrow in the tympanum. Fused to the anterodorsal face of the annulus is a slender arm, the squamosal, which extends towards the posterolateral face of the anterior pterygoid flange and articulates dorsally, by means of a slightly expanded zygomatic ramus, with this element.

A broad articulation exists between the medial base of the compound tympanosquamosal and crista parotica of the occipitopetrosal. The medial base of the tympanosquamosal is cleft anteromedially and the annulus itself is circular with a short otic process, which partially wraps around the pars media plectri of the columella.

Anterior elements

The nasal is a tri-radiate, azygous element with a narrow rostral process, and two semi-lunar shaped lateral components (Illustration 8-1). Posterior margins lie underneath the anterior margin of the frontoparietal, and in all but one specimen, L4, the nasal is connected to the sphenethmoid by means of a well-ossified septum nasi.

The rostral nasal ramus is unusually narrow and parallel-sided, whilst lateral rami are broad, extending well beyond anterolateral margins of the frontoparietal. Bilateral prominences interrupt the most concave region of the anterior nasal profile and are not associated with the septum nasi. The

nasal provides dorsal, mechanical protection to the nasal cavity. Paired, sickle-shaped septomaxillaries support internal cartilages of this cavity, and in some, but not all *X. largeni*, these elements bear a lacrimonasal duct, for passage of sub-ocular tentacles. Lateral margins of septomaxillaries extend almost as far as outer margins of the maxillary arch.

Mandible

The dentary and angulosplenal of the mandible are robust and are separated medially by Meckel's cartilage, which is not continuous across the anterior margin (Illustration 8-3). In no specimen studied was there any medial calcification of Meckel's cartilage. The lateral profile of the mandible is distinctive in being posteriorly-recurved adjacent to the level of the anterior margin of the coronoid flange.

Fusion between cranial elements

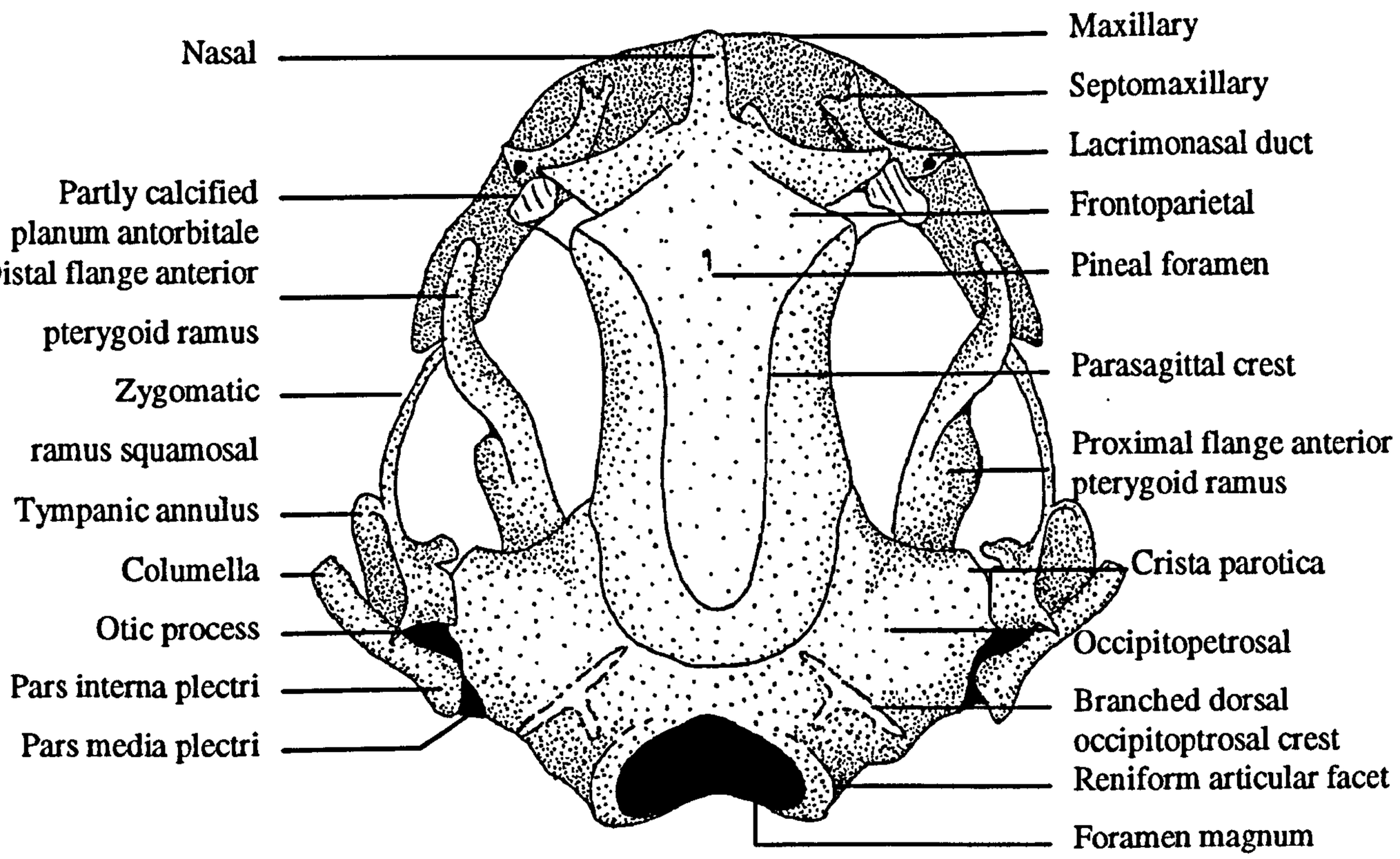
The degree of fusion between various cranial elements varies between specimens and ranges from none to extensive. Fusion may occur between any of the following: frontoparietal and sphenethmoid laterally, frontoparietal and occipitopetrosal, frontoparietal and nasal, sphenethmoid and occipitopetrosal, and rarely, sphenethmoid and vomer.

Calcification of rostral cranial elements

Calcification of cartilage elements ranges from none in some specimens to extensive in others, the degree of which appears to be positively correlated with the extent of fusion (see above). Calcification occurs to varying degrees in the following cartilaginous bodies: septum nasi, rostrally associating nasal cartilage, alary cartilage, nasal cavity cartilage (labeled as element A- [additional chondrified element] in Smirnov, 1994), and the planum antorbitale.

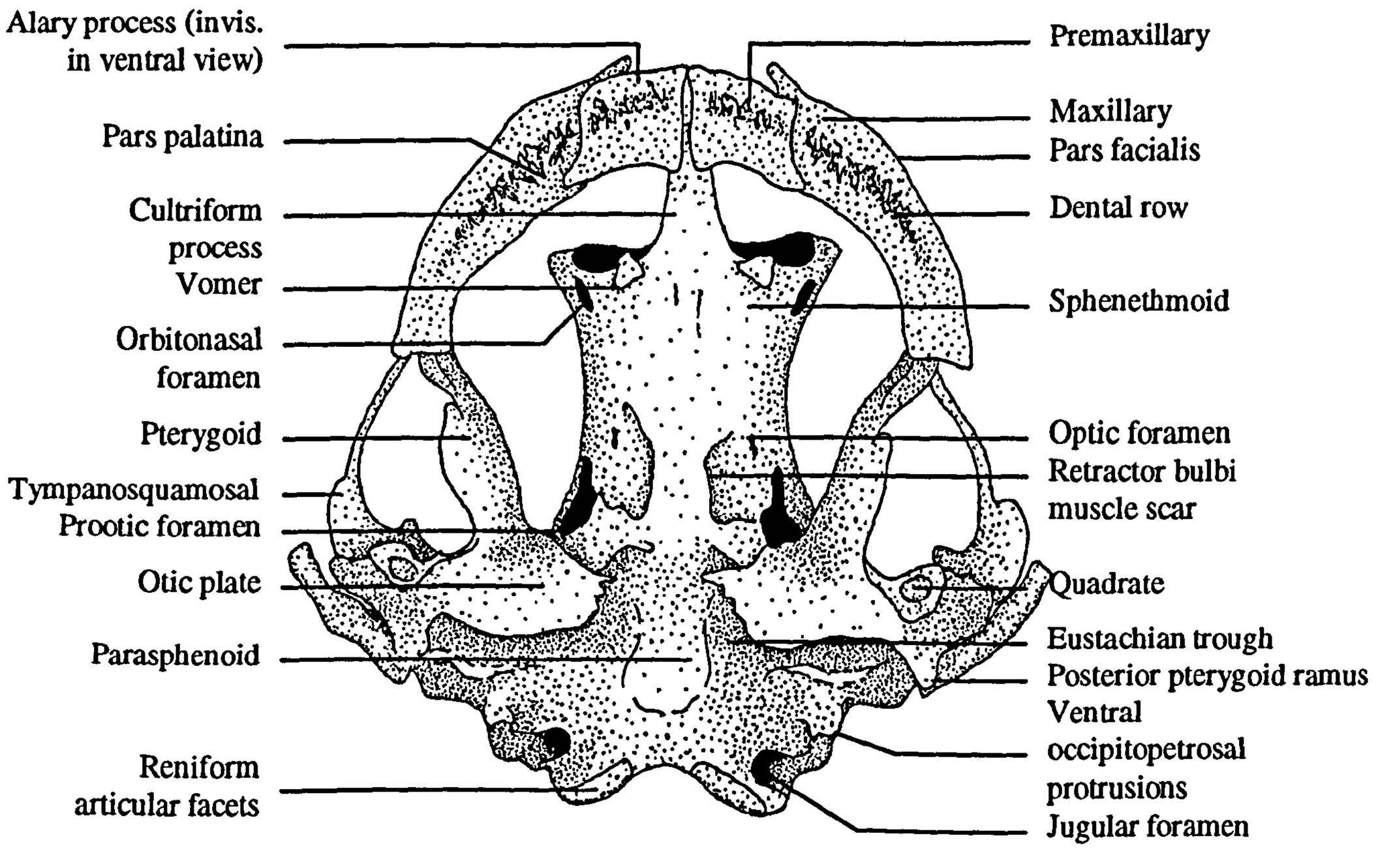
Laryngeal apparatus

In females, the hyolaryngeal apparatus comprises a single pair of rod-like, ossified elements, the posteromedial processes, which converge anteriorly. In males, ossification of the larynx is considerably more extensive. Ossified posteromedial processes become functionally fused anteromedially by extensive calcification of cricoid cartilage. Further calcification results in the formation in some specimens of a posteromedial extension of calcification in the area of cricoid cartilage, which extends along the ventral surface of the element.



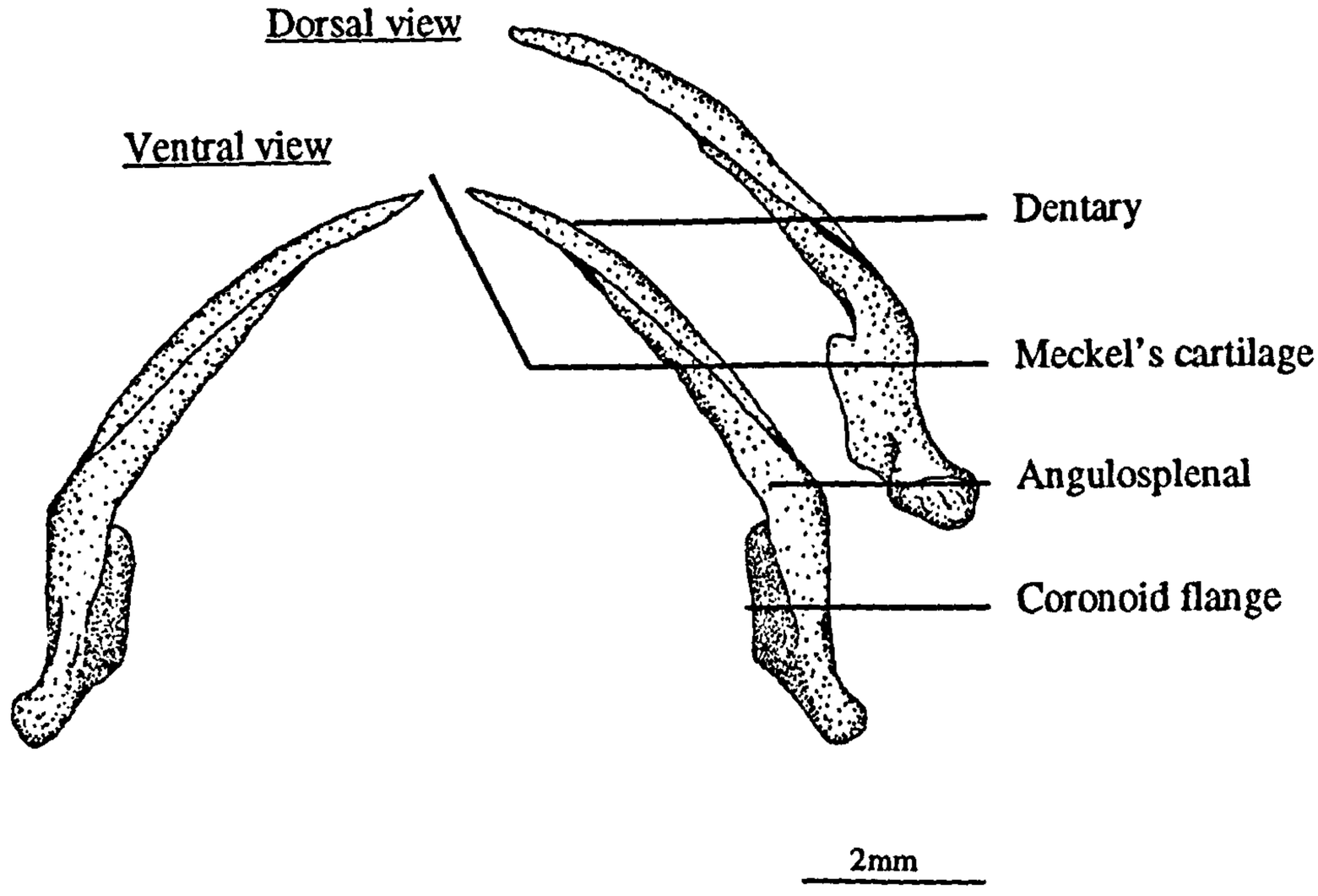
Dorsal view *X. largeni* skull

Illustration 8-1



Ventral view *X. largeni* skull

Illustration 8-2



X. largeni mandible

Illustration 8-3

Axial skeleton

Atlas and axis are fused (Illustration 8-4, Illustration 8-5). This compound vertebral element is short, being equivalent in length to pre-sacral III and bears a pair of small spinal nerve foramina (most are very small, although in L7, foramina are large). The anterodorsal margin of the atlas is flat. Ventrally, a notch separates atlantal cotyles.

Neural arches are non-imbricate, each bearing a single posteriorly-directed process. Vertebrae articulate by means of complex pre- and postzygapophyses. These fluted facets bear ridges and sulci and are present on all pre-sacral vertebrae except for the compound atlas and axis, and occur well within the lateral margins of vertebrae. Zygapophyses are relatively underdeveloped on anterior vertebrae, increasing in complexity and length towards the sacrum. Ribs are ankylosed to tranverse processes, leaving no suture marking the site of fusion.

Each of eight pre-sacral vertebrae bears a pair of transverse processes. Transverse process differ in character between anterior vertebrae I-IV and vertebrae V-VIII. The first pair of transverse processes is very short and oriented perpendicular to the main axis. They are widest at their mid-length and tapered distally. The second pair is the longest; processes are oriented posterolaterally and are slightly expanded distally. Curvature is minimal and restricted to the proximal fifth of the length of the process. The third pair is similar in all respects to the second, but is marginally shorter. Transverse processes on remaining pre-sacrals are considerably shorter, parallel-sided or tapered and are oriented anterolaterally, increasing in angle with respect to the main axial axis, but decreasing slightly in length anteriorly.

Vertebrae decrease in length in the order III, IV, (V, VI, I&II), VII, VIII. Paired sacral diapophyses form from fused IXth and Xth vertebrae, as evidenced by the presence of two pairs of spinal nerve foramina, the first pair being considerably larger than the posteriorly adjacent pair. These diapophyses extend perpendicularly from the axis and are greatly expanded both in anterior and posterior directions, have parallel sides and form a sliding articulation with iliac processes to enable fore-aft movement amounting to 15% of the total snout-vent length (pers. obs.). In three of the seven specimens examined, the posterolateral margins of these diapophyses are recurved ventrally to accommodate the iliac ridges. Remaining vertebrae are fused to the sacrum and form a long rod-like urostyle that extends towards the pelvic girdle base.

Anterior appendicular skeleton

Pectoral girdle

The shaft of the fused scapula-clavicle is particularly robust laterally and is sub-circular in cross section (Illustration 8-6). The clavicle arches anteromedially, becoming flat in cross-section towards the widened midline. A shallow trough extends along the anterodorsal margin for

accommodation of the epicoracoid cartilage and a narrow gap separates parallel, medial margins of these elements. The degree of medial widening of the clavicle is positively correlated with calcification of epicoracoid cartilage in this region.

The scapula is a robust, arched element with a convex anterior margin, concave posterior margin and a greatly elongated posterolateral process. A prominent process on the lateral face of the scapula articulates with the humerus epicondyle. Partes acromialis and glenoidialis are fully fused and there is no evidence of scarring at the site of fusion between these two.

Coracoids are also robust. The shaft is expanded both proximally and distally, and is narrowest medial to the glenoid expansion. These elements demonstrate an unusual degree of medial expansion, with medial margins measuring over twice the width of the element at its glenoid end and almost a third the length of the element. Partial calcification of the cartilage that connects coracoids to the pars glenoidialis results in functional continuity between these elements in most specimens.

The mean angle formed between the zonal axis and coracoid is 48° (s.d.2.8, n=7). Coracoids are separated medially by a moderately wide gap and medial margins are oriented at approximately 45° with respect to each other.

Fused suprascapula and cleithrum are distally bifurcate, the smaller, anterior of the two blades is strongly curved anteromedially. The broader of the two blades is straight and extends medially where suprascapula cartilages overlap slightly. This compound element has a well-developed groove extending from the inner scapular margin along approximately half of the anterior and posterior margins marking the insertion position of suprascapular cartilage into this element.

Epicoracoid cartilages are neither fused to posterolateral margins of coracoids nor to the sternum. The cartilage abuts along the length of the zonal region of the girdle, and is thus functionally firmisternal.

Forelimb

The humerus, or proximal propodial element, articulates (proximally) with the lateral face of the scapula and distally with the compound, epipodial radio-ulna. The humerus in *X. largeni* is particularly robust, especially at its distal association with the radio-ulnare. The lateral epicondyle bears a deep crater into which a process on the lateral face of the scapula inserts during forward movement of the limb. A deep furrow that extends along the proximal third of the element separates proliferated medial and lateral crista. A sulcus extends medially along approximately half the length of the element from troughs that form on both medial and lateral faces of the distal condyle.

The radio-ulna bears a sulcus that extends from the distal margin along approximately half its length and an extended olecranon that curves around the distal humeral condyle. A small sesamoid element is embedded in the ligament connecting these elements.

Carpus

The ulnare lies proximal and post axial to the ulna, and adjacent to the radiale Element Y and the small prepollex are positioned on the preaxial axis. The remaining carpal elements, in post- to pre-axial sequence, are: carpal elements four, three, two and one which lie adjacent to the bases of metacarpals 1-4 respectively. The phalangeal formula is 2-2-3-3. A single sesamoid lies adjacent to the distal end of the radius, along the preaxial axis.

Posterior appendicular skeleton

Pelvic girdle

The pelvic girdle comprises paired ilia, ischia and pubic elements. Ilia extend anteriorly from the acetabular region. These processes bear well-developed dorsolaterally oriented crests along the distal third of their length for articulation with sacral diapophyses, and dorsal processes that are positioned lateral to the narrow, U-shaped symphysis and provide a site for muscle attachment. Proximally, ilia are incorporated into the acetabular region, along with ischia and pubic elements to form a waisted socket that accommodates the femur head. Ischia are medially fused and form a dorsomedial crest and posterior margin of the acetabulum. Pubic elements are fully ossified and are incorporated ventrally into the acetabulum. Anteroventrally, pubic elements give rise to a diamond-shaped epipubis.

Hindlimb

The hindlimb is formed by the weakly-sigmoid femur which articulates proximally with the acetabular region of the pelvic girdle by means of a ball and socket joint, and distally with the condyle of the shank, or compound tibia-fibula. The tibia-fibula articulates with the tibiale and fibulare, which are fused at proximal and distal margins.

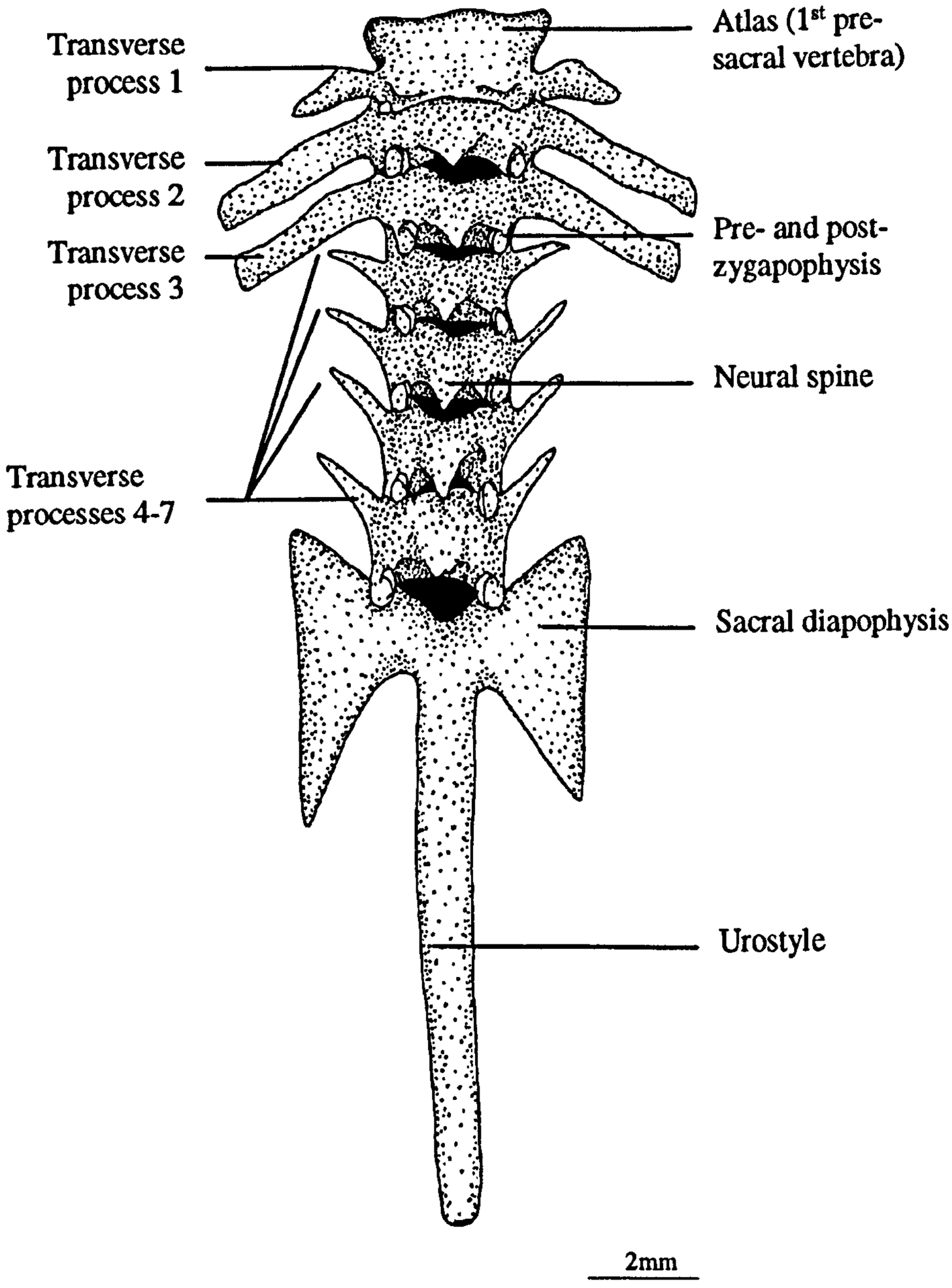
The femur of *X. largeni* is a simple element, which bears a modestly proportioned trochanter (*sensu* Duellman and Trueb, 1986) posteromedially on the proximal end of the shaft.

Likewise, the compound fibula-tibia is simple in structure, lacks any distinguishing crests and has sulci extending from both proximal and distal ends that are almost continuous. In almost all specimens examined, the tibiale and fibulare are fully fused at both ends and lack any distinguishable crests.

Tarsus

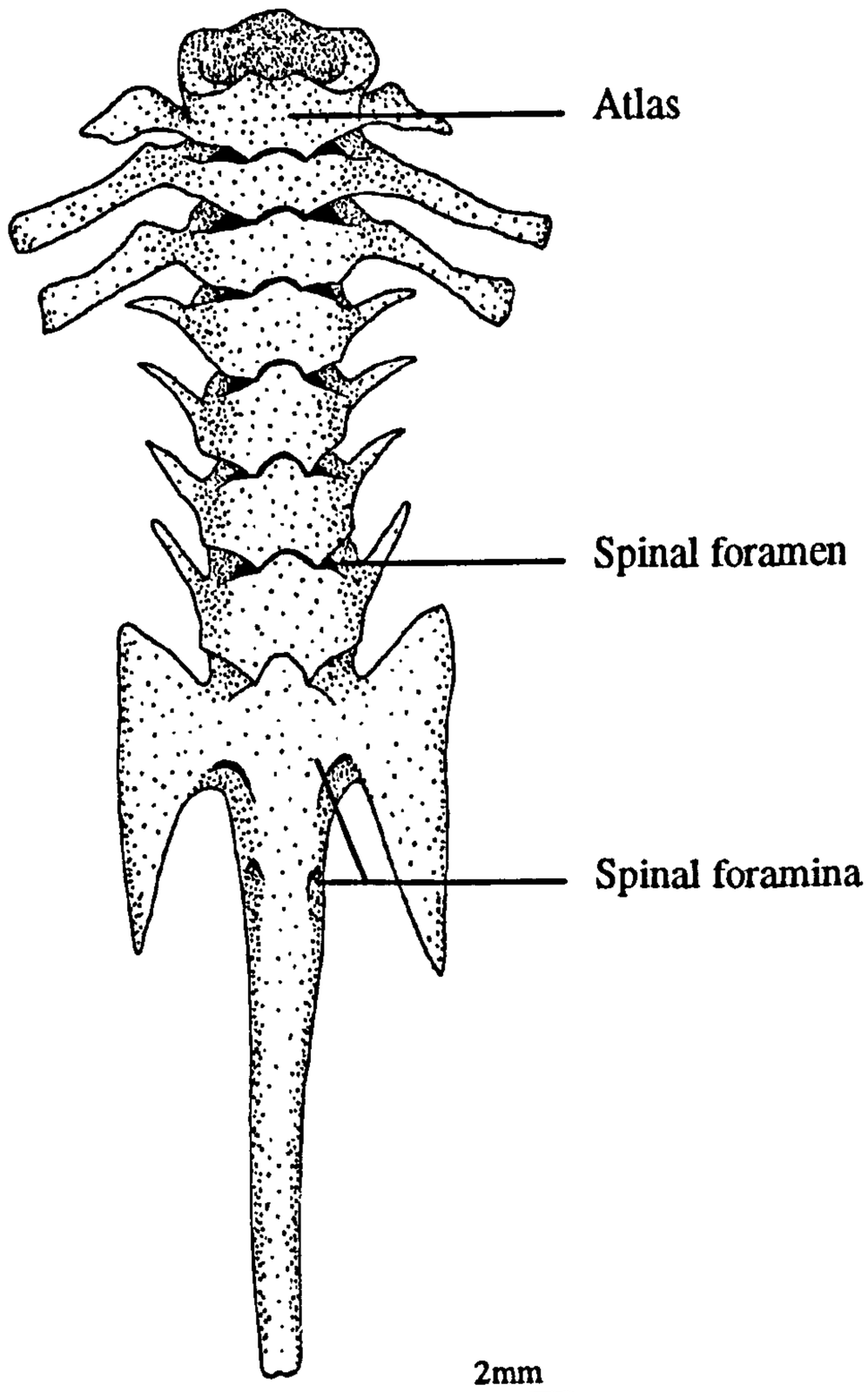
Metatarsals IV and V articulate directly with the distal end of the fibulare postaxially, and there are two distal tarsals: compound distal tarsal 3 and 2, which are fused and articulate with metatarsals II and III, and distal tarsal 1 which articulates with metatarsals I and II (Illustration 8-7). Element Y, related to the tibiale, is positioned dorsal to paired prehallical elements.

The phalangeal formula for is 2-2-3-4-3. Inter-specific variation in tarsal elements is limited, but includes differences in the relative lengths of proximal and distal prehallical elements. The proximal element measures between 2 and 3.5 times the length of the distal element.



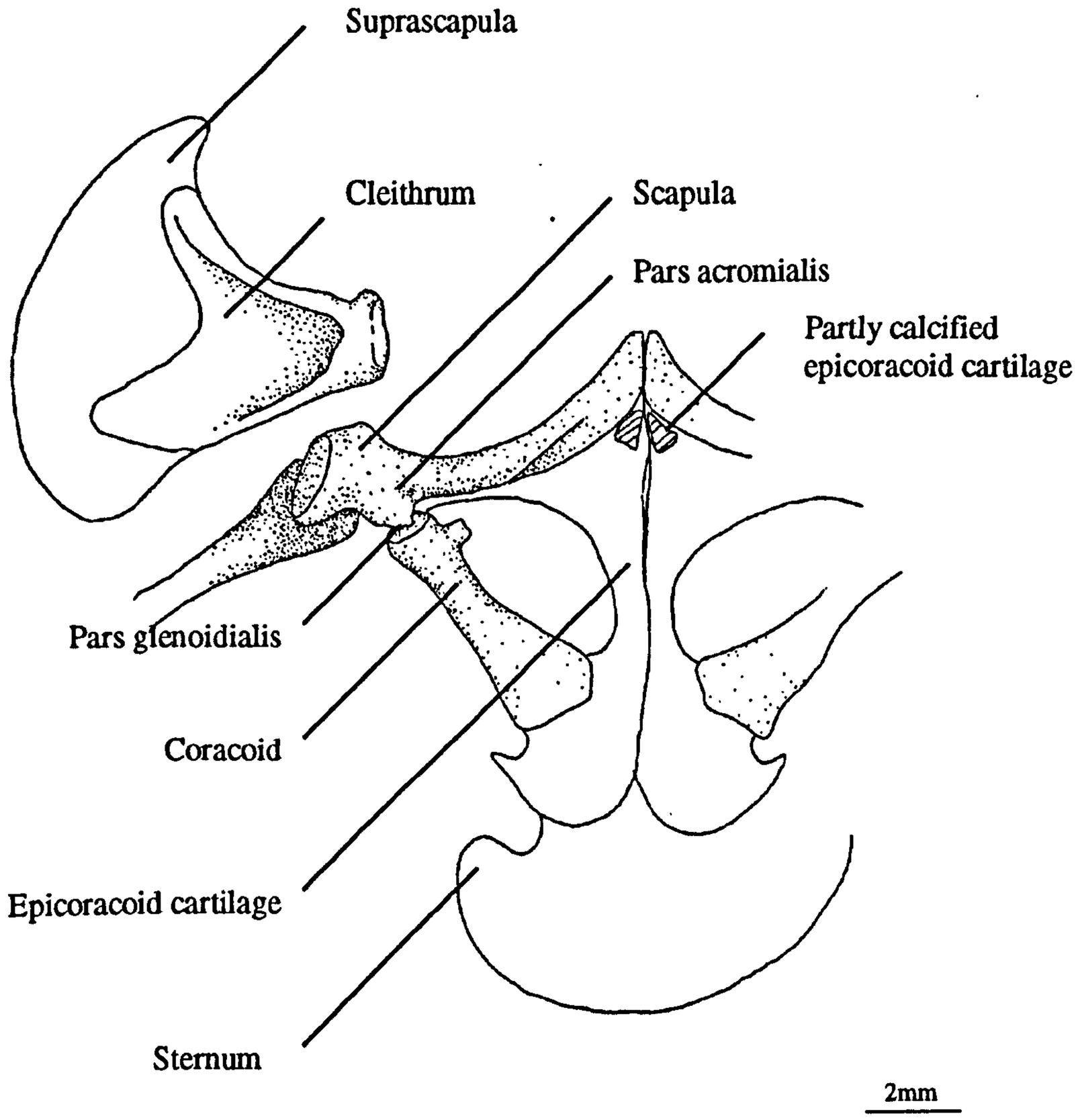
Vertebral view *X. largeni* column dorsal

Illustration 8-4



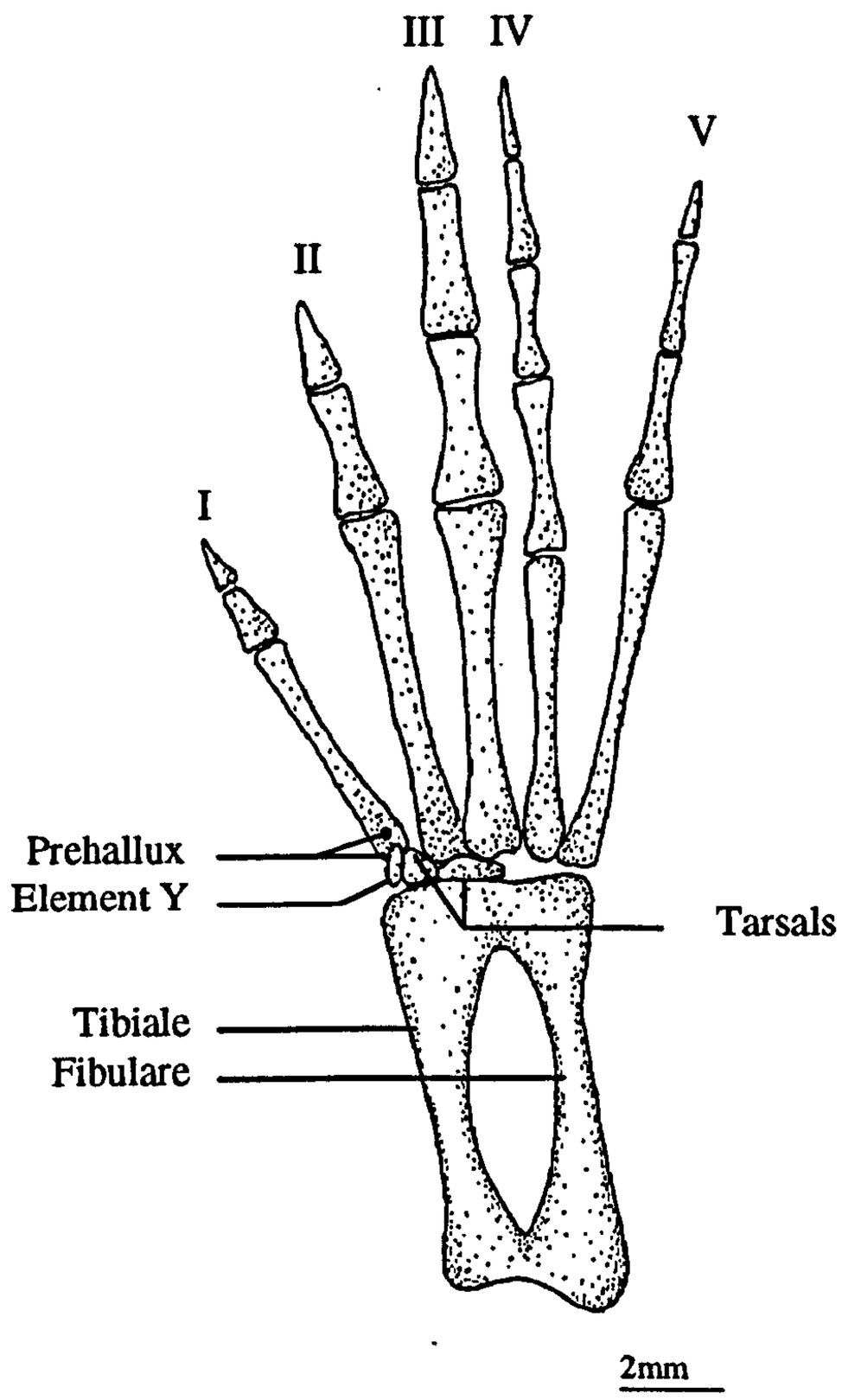
Ventral view *X. largeni* vertebral column

Illustration 8-5



X. largeni pectoral girdle

Illustration 8-6



Tarsus and metatarsals *X. largeni*

Illustration 8-7

DISCUSSION

Inter-specific comparison

A number of features of the sphenethmoid distinguish *X. largeni* from other *Xenopus* taxa. Most notable are the paired vomerine elements. These are small, triangular, azygous elements, widely separated at the bottom of an unusually broad-based cultriform process. *Xenopus* has repeatedly been reported as having an azygous vomer (Noble, 1931; Paterson, 1939; Reumer, 1985; Trueb, 1993; Trueb, 1996) which is considered a synapomorphy of the genus (Cannatella and Trueb, 1988a,b; Cannatella and De Sa, 1993; Baez and Trueb, 1997; Baez and Rage, 1998). This view prevails despite a series of accounts having described a paired state in a number of *Xenopus* taxa, including a single specimen each of *X. laevis* (see Smirnov, 1994), *X. largeni* (see Baez and Trueb, 1997), and 'occasionally' in *X. fraseri, X. borealis* and *X. largeni* (see Baez and Rage, 1998). The present study has demonstrated that whilst vomers are generally azygous, exceptions to this have been found for all taxa examined, the paired state being more common in some species than in others. However, no other taxon demonstrates this state exclusively nor in the same distinctive form as does *X. largeni.*

Furthermore, orbitonasal foramina are entirely enclosed by the sphenethmoid. Although seen occasionally in *X. fraseri* and *X. wittei* (pers. obs.), this character state has not been demonstrated as occurring exclusively in any other taxon. Rtbs are also highly pronounced and optic foramina unusually small in this species. Universal in *X. largeni*, the short crests that extend perpendicularly from dorsal diagonal crests of occipitopetrosal are only otherwise occasionally found in specimens of *X. muelleri* (east).

The maxillary arch is particularly robust in *X. largeni*, with teeth arranged in a disordered manner along the dental row. Similarly, the mandible is remarkably robust, and is distinctly recurved in the region of the coronoid flange. The alary process lacks the distinctly forked lateral margin of other *Xenopus* taxa, instead being evenly rounded.

The pterygoid is distinct in that the ventromedially directed medial flange is cleft, a state occasionally seen in *X. muelleri* (east). Medial margins of otic plates are distinctively rounded rather than being bifid. Foramina appear in septomaxillaries of the majority *X. largeni* specimens, an uncommon anomaly amongst other taxa. The clavicle and coracoid of the pectoral girdle are more robust than in other taxa, each with greatly expanded medial margins. Whilst this is an unusual state for coracoids, clavicles resemble the state seen in *X. laevis*. The pelvic girdle is narrower than in any other taxon examined during the course of the current study, and forms a narrow V-shaped inter-iliac symphysis. Fused atlas and axis distinguish *X. largeni* unambiguously from other *Xenopus* taxa. Recurvature of the posteroventral margins of sacral diapophyses, although not universal in *X. largeni*, has not been observed in any other taxon examined during this study, nor reported in the literature.

Finally, the relative proportions of proximal and distal pre-hallical elements are distinct from those of any other *Xenopus* taxon.

Inter-generic affinities

The osteology of *X. largeni* is profoundly different, in many respects, from that of all other *Xenopus* taxa examined during this study. The scale of inter-specific variation necessitated the additional consideration of information from the literature regarding osteology in *Silurana*, other pipids and closely related non-pipids. Listed below are the main features that distinguish *X. largeni* from all other *Xenopus* taxa examined, unless specified. This is followed by a section that deals with affinities with non-*Xenopus* taxa.

Whilst the state for vomers in extant pipids is variable, with *Silurana*, *Hymenochirus*, *Pseudhymenochirus* and *Pipa* having lost the vomer altogether (Cannatella and Trueb, 1988b; Baez and Trueb, 1997), a paired condition characterizes many other primitive Anura (extant and extinct), namely *Discoglossus, Pelobates, Rhinophrynus, Palaeobatrachus* (see Jurgens, 1971; Cannatella and Trueb, 1988a; Baez and Trueb, 1997).

In *X. largeni,* orbitonasal foramina are enclosed by bone, a state observed in a number of other closely related taxa, including three recently described *Pipa* species (see Cannatella and Trueb, 1988a; Trueb and Massemin, 2001; Trueb, Pugener and Maglia, 2000), *Spea*, *Rhinophrynidae* (see Cannatella and Trueb, 1988b; Trueb, 1996) and a number of fossil pipids *(X. romeri* [see Estes, 1975], *Shelania pascuali* [see Baez and Trueb, 1997]). The state in *Silurana*, *Hymenochirus* and *Pseudhymenochirus* is one of partial enclosure (Cannatella and Trueb, 1988a,b).

A number of character states distinctive to *X. largeni* have also been described for *Silurana*. The very broad-based cultriform process, and gross proportions of the sphenethmoid and its associated posterolateral alae, more closely resemble the states seen in *Silurana* than in *Xenopus* (see Cannatella and Trueb, 1988a,b; pers. obs.). The short crests, oriented perpendicularly to the main dorsal occipitopetrosal crests occur also in *X. (S.) tropicalis* (pers. obs.). The spatulate, medial pterygoid ramus (otic plate) resembles the state seen in *Silurana* (see Cannatella and Trueb, 1988b; pers. obs). Nasals bear prominences along the most concave part of the anterolateral profile; whilst these occur occasionally in some other *Xenopus* taxa, the nasals of *Silurana* routinely bear such prominences (see Cannatella and Trueb, 1988b; pers. obs.). The septomaxillary lacrimonasal ducts observed in most examined specimens of *X. largeni* occur also in *S. tropicalis* (pers. obs.).

A number of additional *X. largeni* characters are synapomorphies of *Silurana* and other non-*Xenopus* taxa. The robustness of the maxillary arch in *X. largeni* resembles the state seen not only in *Silurana* but also in *Pipa* (see Cannatella and Trueb, 1988b; Trueb and Massemin, 2001; pers. obs.). The mandible of *X. largeni* is considerably more robust than that of any other *Xenopus* taxon examined, or indeed any other pipid mandible recorded in the literature, and the degree of posterior

recurvature seen in the *X. largeni* mandible is greater than that seen in other *Xenopus* taxa or in *Silurana*, resembling instead the state seen in *Hymenochirus, Pseudhymenochirus* and *Pipa* (see Cannatella and Trueb, 1988b; Trueb *et al.*, 2000). Finally, atlas and axis are fused in *X. largeni*, a synapomorphy documented for *Silurana, Hymenochirus and Pipa.*

Evolutionary significance of observed variation

Xenopus largeni displays a range of affinities with different *Xenopus* taxa e.g. shape of the skull table resembles that of *X. muelleri*; length of dental row resembles the state in *X. fraseri* and *X. wittei*; anterolateral frontoparietal alae and medially expanded clavicles resemble the state seen in *X. laevis*. On balance however, *X. largeni* bears no overall resemblance to any single *Xenopus* taxon. These affinities shared with different taxa indicate either the expression, in *X. largeni,* of genetic capacities inherited from a common *Xenopus* stock, differentially expressed in different extant species, or alternatively, multiple cases of convergent evolution. The former theory would be likely to have occurred as a result of early divergence from a basal *Xenopus* stock, whilst the latter, taken in isolation, seems implausible given the range of coincident convergences. It is possible that both processes have been working in parallel during the evolution of *X. largeni.*

Whilst it is indisputable that *X. largeni* belongs to the genus *Xenopus*, a number of its distinguishing characters are shared with no other *Xenopus* taxon, but instead with *Silurana*. Most significant of these is the fused first and second pre-sacral vertebrae, listed as a *Silurana* apomorphy by Trueb and Cannatella (1988a) supporting the theory of an early divergence within the *Xenopus* lineage.

Osteological evidence, taken as a whole, places *X. largeni* in a systematic position that straddles the boundary between *Xenopus* and *Silurana*, implying that the division between these taxa is perhaps not as robust as had previously been suggested by Trueb and Cannatella (1988a,b). A systematically intermediate position, such as this one, has also been suggested for a dodecaploid species from Cameroon, *X. longipes*, also demonstrating a number of *Silurana* apomorphies (Loumont and Kobel, 1991). My personal opinion regarding the osteology of *X. longipes*, albeit based on illustrations alone and on documentation included in the original species account, is that it represents a highly neotenic form, as determined by comparison with juvenile *X. l. laevis* and *X. muelleri* (pers. obs.), and reports from the literature.

Xenopus longipes is documented as having paired nasals, a characteristic which has been observed in juvenile *X. l. laevis* (see Reumer, 1985). Laterally displaced parasagittal crests, thin squamosals, large auditory capsules, tympanosquamosal attached to the anterodorsal surface of occipitopatrosals and straight transverse processes are all characteristics that I associate with an underdeveloped state or with *Xenopus* taxa in which certain characters develop along a neotenic ontogenetic trajectory (e.g. *X. fraseri* transverse processes, parasagittal crests; pers. obs.). It is

unlikely, on the basis of these osteological characters that *X. longipes* belongs to a 'hitherto unknown subgroup of *Xenopus*' as was suggested by Loumont and Kobel (1991). I do not doubt, however, that the taxonomic position of *X. longipes* falls between that of *Xenopus* and *Silurana*, since specimens that formed the basis of the original osteological account were sexually mature, and possessed a number of non-osteological characters that suggest an intermediate position. However, all of the osteological characters said to distinguish *X. longipes* from *Xenopus sensu stricto* I consider to be ontogenetically variable, and none is shared with *X. largeni*.

Of considerable interest is that keratinized spinules, documented only for *X. longipes* and members of the sub-genus *Silurana*, were also found on one of the three *X. clivii* specimens loaned by the BMNH for x-ray, and is strongly suggestive that specimens currently labeled as *X. clivii* may comprise more than one species, at least one of which represents a taxonomically-intermediate form with respect to *Xenopus* and *Silurana*.

Xenopus largeni also demonstrates a number of character states otherwise found only in non-*Xenopus* taxa. Whilst it is possible that this has occurred as a result of convergent evolution, it is perhaps more plausible that it is the result of the expression of genetic capacities, silenced or latent in the most closely related taxa, but shared with a more basal common ancestor.

Whilst not exhaustive, literature on the osteology of pipids and related Anura is relatively comprehensive, and represents a wide variety of forms. Still, it has not been possible to trace a number of character states found in *X. largeni* to any other taxon. These include position of the pineal foramen, paired vomer, disordered dental row and cleft medial margin of tympanosquamosal. As such, they appear to represent uniquely derived states.

Despite the small sample size and unequal sex ratio, the lack of intra-specific variation in characters listed as distinguishing *X. largeni* from other *Xenopus* taxa (albeit all osteologically prepared specimens were siblings, but X-rays provided some additional information regarding non-related specimens) suggests that *X. largeni* straddles the boundary between *Xenopus* and *Silurana*, and probably diverged at an early date from remaining *Xenopus* stock. This is supported by results from x-rays that also show un-fused atlas and axis non-related *X. largeni* specimens. The high number of affinities with non-pipids further supports evidence for an early divergence. More fieldwork is needed in order to increase this sample size since all available material has been exhausted for the purpose of the present study.

CONCLUSION

A comprehensive account describing the osteology of *X. largeni* is presented for the first time. Results from inter-species comparisons are suggestive of a long period of genetic isolation, in line with evidence from other studies which show the fauna and flora of highland Ethiopia to have

long been isolated from those of other highland areas in Africa: avifauna (Moreau, 1966); flora (Hedberg, 1957); trees and shrubs (Hamilton, 1982); mammals (Yalden *et al.*, 1996); other amphibians (Largen and Drewes, 1989; Lovett, 1993; Largen, 1998, 2001) avifauna. Isolation would have been exacerbated by its environmental history and geography. The degree of osteological differentiation separating *X. largeni* from other *Xenopus* taxa, along with evidence of shared affinities with other pipids, indicates that this period of isolation has probably persisted for longer than the Pleistocene (2.5 million yrs). Indeed, if one combines osteological evidence that shows *X. largeni* to straddle the taxonomic boundary between *Silurana* and *Xenopus* with published dates documenting the major *Silurana-Xenopus* split as having occurred as many as 120Mya (Knochel, Korge, Basner, Meyerhoff, 1986), it seems possible that this Ethiopian taxon diverged from basal *Xenopus* stock at the same approximate period.

Owing to the poor quality of x-ray images of *X. clivii* and the lack of available material for osteological preparation, it was not possible to determine whether the osteology of *X. clivii* parallels that of *X. largeni* in terms of indicating a prolonged period of genetic isolation. Since no such information exists in the literature, and results from a range of molecular and biochemical studies are yet to ascertain precise relations for this species (Graf, 1996; Kobel *et al.*, 1998), further fieldwork remains the only option if comparative osteology is to be applied as a means of determining its phylogenetic status.

BIBLIOGRAPHY

Bàez, A.M. and Pugener, L.A. (1998). A new Palaeogene pipid frog from northwestern Patagonia. *Journal of Vertebrate Palaeontology* **18(3)**:511-524.

Bàez, A.M. and Rage, J.C. (1998). Pipid frogs from the upper Cretaceous of In Beceten, Niger. *Palaeontology* **41 (4)**:669-691.

Bàez, A.M. and Trueb, L. (1997). Redescription of the Paleogene *Shelania pascuali* from Patagonia and its bearing on the relationships of fossil and recent pipoid frogs. *Scientific papers, Natural History Museum, University of Kansas* 4:1-41.

Bidgood, S. and Friis, I. (1998). *Sespania melanocaulis*, sp. Nov. (Leguminosae subfam. Papilionoidea) from SW Ethiopia. *Nordic journal of Botany* **18(2)**:209-213.

Brown, D.S. (2001). Taxonomy, biogeography and phylogeny of the non-lacustrine African freshwater snails belonging to the genera *Ceratophallus* and *Afrogyrus* (Mollusca: Planorbidae). *Journal of Zoology, London* **255**:55-82.

Burki, E. and Fischberg, M. (1985). Evolution of globin expression in the genus *Xenopus* (Anura: Pipidae). *Molecular Biology and Evolution* **2**:270-277.

Burki, E., Schwager, J. and Fischberg, M. (1984). Electrophoretic patterns of haemoglobin in different *Xenopus* species, subsepecies and interspecies hybrids. *Experientia* **40**: 59-64.

Cannatella, D.C. and De Sá, R.O. (1993). *Xenopus laevis* as a model organism. *Systematic Biology* **42**:476-505.

Cannatella, D.C. and Trueb, L. (1988a). Evolution of pipoid frogs: inter-generic relationships of the aquatic frog family Pipidae (Anura). *Zoological Journal of the Linnean Society* **94**:1-38.

Cannatella, D.C. (1988b). Evolution of pipoid frogs: Morphology and phylogenetic relationships of *Pseudhymenochirus*. *Journal of Herpetology* **22** (4):439-456.

Carcasson, R.H. (1964). A preliminary survey of the zoogeography of African butterflies. *East Africa wildlife journal* **2**:122-157

de Klerk, H.M., Crowe, T.M., Fjeldsa, J. and Burgess, N.D. (2002). Patterns of species richness and narrow endemism of terrestrial bird species in the Afrotropical region. *Journal of Zoology, London* **256**:327-342.

Estes, R. (1975c). Relationships of the South African fossil frog *Eoxenopoides reuningi* (Anura, Pipidae). *Annals of the South African Museum* **73**:49-80.

Fjeldsa, J., Ehrlich, D., Lambin, E. and Prins, E. (1997). Are biodiversity 'hotspots' correlated with current ecoclimatic stability? A pilot study using the NOAA-AVHRR remote sensing data. *Biodiversity and Conservation* **6**:401-422.

Fjeldsa, J. (1997). Geographical patterns of old and young species in African forest biota: the significance of specific montane areas as evolutionary centres. *Biodiversity and Conservation* **6**:325-346.

Fjeldsa J. and Lovett, L.C. (1997). Biodiversity and environmental stability. *Biodiversity and Conservation* **6**:315-323

Graf, J.-D. and Fischberg, M. (1986). Albumin evolution in polyploid species of the genus *Xenopus*. *Biochemistry and Genetics* **24**: 821-837.

Graf, J.-D. 1996. "Molecular approaches to the phylogeny of *Xenopus*". Pp.379-388 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Hamilton, A.C. 1972. "The interpretation of pollen diagrams from highland Uganda." Pp. 46-149 in Palaeoecology of Africa, The Surrounding Islands and Antarctica. 1st ed., edited by van Zinderen Bakker Sr., E.M. Cape Town: Balkema.

Hamilton, A. (1976). The significance of patterns of distribution shown by forest plants and animals in tropical Africa for the reconstruction of upper Pleistocene Palaeoenvironments: A Review. *Palaeoecology of Africa* **9**:63-97.

Hamilton, A.C. ed. 1982. Environmental History of Africa: A Study of the Quaternary. London: Academic Press.

Hedberg, O. (1957). Afroalpine vascular plants. *Symbolae Botanicae Upsalienses* **15**: 1-411.

Hedberg, O. (1969). Evolution and speciation in a tropical high mountain flora. *Biological Journal of the Linnean Society* **1**:135-148.

Jurgens, J. (1971). The morphology of the nasal region of Amphibia and its bearing on the phylogeny of the group. *Annals of the University of Stellenbosch* **46, Serial A, 2**:1-146.

Knochel, W., Karge, E., Basner, A. and Meyerhof, W. (1986). Globin evolution in the genus *Xenopus*: Comparative analysis of cDNAs coding for adult globin polypeptides of *X. borealis* and *X. tropicalis*. *Journal of Molecular Evolution* **23**:211-223.

Kobel, H.R. 1996. "Allopolyploid speciation." Pp391-400 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Kobel, H.R., Loumont, C. and Tinsley, R.C. (1996). "The extant species." Pp 9-33 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Kobel, H.R., Barandun, B. and Thiébaud, Ch.H. (1998). Mitochondrial rDNA phylogeny in *Xenopus*. *Herpetological Journal* **8 (1)**:13-18.

Largen, M.J. (1998). A preliminary review of the amphibians of Ethiopia. *The Herpetological Journal* **8(1)**:7-12.

Largen, M.J. (2001). Catalogue of the amphibians of Ethiopia including a key for their identification. *Tropical Zoology* **14(2)**:307-402.

Largen, M.J. and Drewes, R.C. (1989). A new genus and species of brevicipitine frog (Amphibia, Anura, Microhylidae) from high altitude in the mountains of Ethiopia. *Tropical Zoology* **2**:13-30

Loumont, C. and Kobel, H.R. (1991). *Xenopus longipes* sp. nov., a new polyploid pipid from western Cameroon. *Revue Suisse de Zoologie* **98 (4)**:731-738.

Lovett, J.C. 1993. "Climatic history and forest distribution in eastern Africa." Pp. 23-29 in Biogeography and Ecology of the Rainforests of Eastern Africa, edited by Wasser, S.K., and Lovett, J.C. Cambridge: Cambridge University Press.

Mann, W., Risley, M.S., Eckhardt, R.A. and Kasinsky, H.E. (1982) Characterization of sperm/spermatid basic chromosomal proteins in the genus *Xenopus* (Anura: Pipidae). *Journal of Experimental Zoology* **200**:173-186.

Moreau, R.E. (1966). The bird fauna of Africa and its islands. Academic press, New York and London.

Noble, G. 1931. The biology of the Amphibia. McGraw-Hill Book Co., New York.

Olson, D.C., Dinerstein, E., Wikramanayake, E.D., Burgess, N.D., Powell, G.V.N., Underwood, E., D'Amico, J.A., Itoua, I.I., Strand, H.E., Morrison, J.C., Loucks, C.J., Allnutt, T.F., Lamoreux, J.F., Ricketts, T.H., Kura, Y., Lamoreux, J.F., Wettengel, W.W., Hedao, P. and Kassem, K. (2001). Terrestrial ecoregions of the world: a new map of life on earth. *Bioscience* **51(11)**: 933-938.

Paterson, N.F. (1939). The head of *Xenopus laevis*. *Quarterly Journal of Microscopical Science* **81**:161-234.

Reumer, W.F. (1985). Some aspects of the cranial osteology and phylogeny of *Xenopus* (Anura: Pipidae). *Revue Suisse de Zoologie* **92 (4)**:969-980.

Smirnov, S.V. (1994). Postmaturation skull development in *Xenopus laevis* (Anura, Pipidae): Late-appearing bones and their bearing on the pipid ancestral morphology. *Russian Journal of Herpetology* **1, 1**:21-29.

Tinsley, R.C. (1995). A new species of *Xenopus* (Anura: Pipidae) from the highlands of Ethiopia. *Amphibia-Reptilia* **16**.375-388.

Trueb, L. 1993. "Patterns of Cranial Diversity Among the Lissamphibia." Pp. 255-343 in The Skull. Vol. 2, edited by Hanken, J., and Hall, B.K. Chicago: The University of Chicago.

Trueb, L. (1996). "Historical constraints and morphological novelties in the evolution of the skeletal system of pipid frogs (Anura: Pipidae)." Pp. 349-376 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Trueb, L. and Massemin, D. (2001). The osteology and relationships of *Pipa aspera* (Amphibia: Anura: Pipidae), with notes on its natural history in French Guiana. *Amphibia-Reptilia* **22**:33-54.

Trueb, L., Pugener, L.A. and Maglia, A.M. (2000). Ontogeny of the bizarre: An osteological description of *Pipa pipa* (Anura: Pipidae), with an account of skeletal development in the species. *Journal of Morphology* **243**:75-104.

Tymowska, J. 1991. "Polyploidy and cytogenetic variation in frogs of the genus *Xenopus*". Pp. 259-297 in Amphibian cytogenetics and evolution. Green, D.M. & Sessions, D.K., (Eds.) San Diego:Academic Press.

Werger, M.J.A. 1978. "The Karoo-Namib Region." Pp. 231-99 in Biogeography and Ecology of Southern Africa, edited by Werger, M.J.A. The Hague: The Hague:Junk.

Yalden, D.W., Largen, M.J., Koch, D. and Hillman, J.C. (1996). Catalogue of the mammals of Ethiopia and Eritrea. 7. Revised checklist, zoogeography and conservation. *Tropical Zoology* **9** (1):73-164.

SUMMARY AND CONCLUSION

The widespread use of the genus *Xenopus* as a model group in biomedical research has generally been restricted to a single taxon, *X. l. laevis* (Gurdon, 1996). Furthermore, what little is known regarding the ecology of the group is founded primarily on studies that have been conducted on this taxon alone. Relatively little is known with respect to the remaining 16 *Xenopus* species (Kobel, Loumont and Tinsley, 1996). It is known however, that the chromosome complement of all but a single species is polyploid, with only a single taxon representing the diploid state. Chromosome complements vary in remaining taxa between tetraploid, octoploid and dodecaploid (Kobel, 1996), this genus representing the group with greatest variation in ploidy known among vertebrates (Schmidt and Steinlein, 1991), a feature that clearly demonstrates the importance of inter-species hybridization in its evolution.

The genus *Xenopus* is almost entirely aquatic in habit, leaving water only rarely during short migrations to adjacent water bodies (Measey, 1997). The group is able to tolerate prolonged periods of aestivation and starvation and has demonstrated its adaptability and tolerance for living under conditions far removed from those of its current natural distribution by becoming established as feral populations in a number of localities which include north and south America, Ascension Island and South Wales (Tinsley and Kobel, 1996). Unusually for Amphibia, *Xenopus* call underwater using a unique sound production mechanism (Yager, 1996), and use a remarkably precise auditory and lateral line system for locating underwater stimuli (Elapfandt, 1996). Behavioural studies have provided preliminary evidence to indicate territoriality in males (Tinsley and Kobel, 1996).

The genus is Gondwanan in origin, fossils having been dated from the Cretaceous, as early as 90-80MyrBP, from In Beceten, Niger and from northwestern Patagonia, Argentina (Baez, 1996). Currently however, species of the genus are restricted to Africa, their distribution extending throughout Africa, primarily south of the Sahara. Taxa are broadly divisible into forest and non-forest dwelling groups, with individual taxon ranges varying in size from a single mountain to a broad expanse of lowland savanna, extending over a distance of thousands of kilometers.

A resurgence in research interest in the group began in the early 1970's at a time when only six species were known. Their distribution ranges were believed to be largely allopatric, reflecting broad divisions between forest and non-forest dwelling taxa, with the exception of two endemics which are restricted to the Ethiopian highlands and the Cape fynbos respectively (Tinsley, Loumont and Kobel, 1996). With a tripling in the number of species recognized since the beginning of this resurgence (Tinsley and Kobel, 1996), the pattern of distribution, inevitably, has become complicated. Inter-species distinction has also become problematic with cryptic species now appearing to be widespread, making morphological distinction between some taxa difficult, indeed impossible in some cases.

The distribution of *Xenopus* taxa will have undergone extensive and successive periods of remodeling during cyclical climate changes of the Quaternary, at times forcing taxa into sympatry, a situation known to promote hybridization in the genus *Xenopus*. Accepting the premise that the current distribution is likely to reflect constraints imposed by continental conditions during the most recent glacial maximum (see chapter 1), it is pertinent that the foci for endemism and species richness in the genus *Xenopus* match those for other taxon groups (Wasser and Lovett, 1993). Congruence between patterns shown across a range of groups highlights a number of geographical areas as being foci of biogeographical interest. It is these areas, and those currently associated with biogeographical boundaries, that have formed the basis for taxonomic studies on *Xenopus* taxa in the current thesis.

Owing to many of the regions of primary biogeographical interest lying in politically unstable areas, arranging to conduct fieldwork was logistically complicated if not altogether impossible. Where feasible, fieldwork was conducted and studies carried out on living or recently fixed material (e.g. Swaziland and South Africa, chapter 7). In other instances, material was obtained from existing museum and university collections. In the latter event, the methods that could be reliably applied to the determination of taxonomic status of specimens were necessarily limited by the conditions under which these specimens had been fixed and stored. Comparative osteology, though previously used very little to these ends (despite the main systematic divisions within Anura being based on osteology), has previously proven to be highly informative (see Reumer, 1985).

Prior to commencing any comparative inter-species study however, it was imperative that an overview of osteology in the genus *Xenopus* be conducted (chapter 2). Despite only having access to just over half of all recognized *Xenopus* taxa, an overview incorporating this information would serve to eliminate much of the ambiguity and inconsistency which exists in the literature with regard to the osteological characteristics of these taxa and would provide the most comprehensive assessment to date of osteology in the genus. Information was also drawn from the literature, where possible, regarding the osteology of specific taxa that were not available for the current study (e.g. *X. longipes* Loumont and Kobel, 1991). Despite many references having been made in the literature to certain aspects of osteology in the genus, many of these have relied on a limited number of taxa, each represented by a small number of specimens, some of which have been juvenile in form (Baez, 1996). No account exists which describes the form and degree of osteological variation within the genus, and consequently, a high degree of uncertainty surrounds the taxonomic position of certain fossil taxa (Baez, 1996). Results from the current study (chapter 2) provide evidence that the osteological distinction that has previously been drawn between the two *Xenopus* sub-genera (Cannatella and Trueb, 1988) is artificially robust. Results show that at least two of the taxa examined towards this account straddle the taxonomic boundary between these two sub-genera. Furthermore, the study provides information that will facilitate the determination of the taxonomic position of existing and future fossil taxa thereby enabling a reassessment of the former geographical range of the group.

During the course of work carried out towards chapter 2, it became apparent that a previously undocumented degree of intra-specific variation exists which is so great in some instances as to preclude the identification of inter-specifically variable osteological characters. Furthermore, intra-specific variation of some of the characters determined by Reumer (1985) to be taxonomically significant was found to be so great as to preclude their use as unambiguous taxonomic indicators. That intra-specific variation has been largely ignored in previous studies may be the result of a heavy reliance on necessarily small sample sizes. Chapter 3 is therefore dedicated to the determination of the form and degree of intra-specific osteological variation in a single taxon. In an attempt to understand the causal factors behind intra-specific variation, to describe the degree and form of this variation and to identify a set of reliable specific indicators, a sample of *X. l. laevis* was selected so as to represent the maximum available age- and size-range of both sexes. Whilst it was not possible to distinguish between the variable effects of size, age and sex on osteology, the study identified a core set of features that characterize the taxon, and do not appear to vary intra-specifically. An apparent sex-biased skew in age-range was identified which suggested that females live for longer than males, corroborating earlier such observations by R. Tinsley (pers. com.). Further investigations are required to confirm this as a natural phenomenon. Results from the aspect of the study concerned with age-variation differed significantly from those published in an earlier study by Smirnov (1994) who identified a small number of characters in *X. l. laevis* that were said to vary with age. Vomers were said to be azygous in juveniles, becoming paired later in life, ultimately fusing to the sphenethmoid. Similarly, the palatine element was said to be absent in juveniles, appearing later in life prior to fusing to the cultriform process. The parasphenoid was said to develop lateral extensions later in life and mentomeckelian ossifications said to develop between paired mandibles. A pre-orbital process was also said to develop on the dorsal surface of the maxillary. The current study, which was based on a wild-caught sample of 34, as compared with 8 lab-reared specimens used in Smirnov's study, found that whilst the vomer was generally azygous, it was occasionally paired. Variation in this character was not determined to be age-related. Likewise, no palatine elements were found in any specimen, neither did the parasphenoid develop lateral extensions, nor a mentomeckelian ossification appear at any stage of post- maturation development, nor, finally, a pre-orbital process appear on the maxillary. Repeated requests to borrow the material used in Smirnov's study proved fruitless. Inconsistencies identified between these two sets of results may be a consequence of differences in sample size and/or rearing conditions.

Whilst the features identified in the current study as characterizing the taxon do not necessarily extend to other taxa in the genus, these findings taken as a whole emphasize a need for care when ascribing certain character states to any single taxon without having assessed a representative range of specimens.

Having established a basic level of understanding and familiarity with osteology in the genus *Xenopus*, the focus of my work could now shift to specific biogeographical areas and situations of taxonomic interest. Two chapters (chapter 4 and 5) focus on the central African highlands, long recognized as an area of significant biogeographical interest, being a focus for endemism, species diversity and for species showing a disjunct distribution across a range of taxa (Wasser and Lovett, 1993; DeKlerk, Crowe, Fjeldsa and Burgess, 2002). These highlands are postulated to have been an ice-age refugium to a range of forest taxa during periods of aridity corresponding to successive periods of ice-sheet expansion over parts of the Northern Hemisphere during the Pleistocene. It is likely that conditions of sympatry, as would have existed in refugia, will have promoted hybridization in *Xenopus*.

Two octoploids, *X. wittei* and *X. vestitus*, living in these highlands are postulated to be the allopolyploid product of hybridization between the predecessors of two tetraploid taxa, *X. fraseri aff.* and *X. l. victorianus*, whose ranges are known to come into contact in a number of sites in this area (Tinsley, 1981), and are likely to have done so repeatedly throughout the Pleistocene. Comparative osteology was used to assess interrelatedness between these four taxa. Results largely correspond to those obtained in previous molecular, biochemical, parasitological, mtDNA and mtRNA studies (Tymowska, 1977; Burki and Fischberg, 1985; Carr, Brothers and Wilson, 1987; Tinsley, 1996; Kobel, Barandun and Thiebaud, 1998; Tinsley and Jackson, 1998). Congruence between results from the current study and previous studies qualifies application of this method for taxonomic investigation at the species/ sub-species level, and implicates both parent species in the hybrid origin of each octoploid species, though evidence indicates that the event that gave rise to *X. vestitus* is likely to have occurred at a much earlier stage of the Pleistocene, if not altogether preceding this geological period.

Chapter 5 focuses on two morphologically cryptic taxa, *X. l. bunyoniensis* and *X. l. victorianus*. The former is reported as having been endemic to the interlacustrine highlands of Central Africa, literature records indicating a local abundance of this taxon early during the last century (Tinsley, 1973, 1981; Tinsley, Kobel and Fischberg, 1979). However, *X. l. bunyoniensis* has not been found there for over thirty years, apparently having been replaced by one of three other taxa: the more widespread taxon, *X. l. victorianus*, whose distribution extends from these highlands and the eastern Democratic Republic of the Congo eastwards as far as Lake Victoria, and endemics *X. wittei* and *X. vestitus* (Tinsley, 1981). The museum record enables the determination of the precise period of this turnover event, in one locality at least, to the late 1950's (Tinsley, 1981). It is unusual that a replacement event should be so well documented. However, a compilation of the accounts that document these events (see chapter 5) appears to cast doubt on the validity of the taxonomic distinction between *X. l. victorianus* and *X. l. bunyoniensis*, and ultimately on the claim that *X. l.*

bunyoniensis was replaced by the former taxon in regions within these highlands. Owing to this ambiguity, existing evidence for the distinctness of these two taxa was assessed and a study of comparative osteology conducted. It would appear that much of the existing evidence of taxonomic distinction between the two could be discounted on grounds of methodological inconsistencies, natural physiological variation and the probable effects of parasite infection and malnutrition. However, results from the study of comparative osteology reveal significant variation between the two, mainly with regard to upper jaw morphology. Though statistically significant, the systematic significance of these differences is unknown. Whilst the effects of malnutrition on osteology have not yet been determined for the genus, it is possible that these may be responsible for the observed variation.

Moving away from the Central African highlands, chapter 6 looks at a situation that has received considerable attention in recent years. *Xenopus muelleri*, formerly believed to have the widest distribution range of all *Xenopus* taxa, is now believed to comprise two sibling taxa, an eastern and a western form. This claim is based on evidence of parasitology and biochemistry (Kobel, Loumont and Tinsley, 1996), and most recently of rDNA (Kobel, Barandun and Thiebaud, 1998). Studies to date have compared evidence from samples collected from parts of the distribution range that lie in and to the west of Cameroon with those collected from Tanzania. Whilst results from these studies are unambiguous, no attempt has yet been made to determine whether this variation represents a polytypic cline or whether the taxon currently referred to as *X. muelleri* comprises two distinct forms whose ranges meet at an intervening part of the range. The current study of comparative osteology compared *X. muelleri* collected from the far southeastern part of its range with a sample from the northwestern-most part of its range and found samples to differ significantly from one another. A number of samples from intervening localities were subsequently incorporated for comparison and revealed an unexpected pattern of variation between samples. Indeed eastern samples differed from those in the west, the geographic boundary between these forms appearing to lie near the northern part of Lake Tanganyika, but a high degree of variation also separated each of the eastern samples from one another. Specifically, samples separated by 50km were as osteologically distinct from one another as each was from a sample collected from a site thousands of km distant. Re-examination of osteology in the sample, in the light of results from chapters 2 and 3, provided an unexpected perspective, leading to a preliminary explanation for this pattern of variation in terms of there having been extensive and widespread hybridization between *X. l. laevis* and *X. muelleri* in eastern parts of the *X. muelleri* range. The following chapter offered corroboration for this interpretation.

Chapter 7 was based on material collected live during a period of fieldwork in southeastern Africa and in Swaziland, and found hybridization between *X. l. laevis* and *X. muelleri* to be

widespread across a transect that intersects the margins of their respective distribution ranges and is widely recognized as an area of significant biogeographical interest (Poynton and Boycott, 1996). Prior to this study, reports of natural hybridization between these taxa were limited to a single case, a sterile F1-hybrid reported by Fischer, Koch and Elepfandt (2000). This morphologically intermediate specimen was found to be diploid with respect to *X. muelleri* and *X. l. laevis*, expressing dual inheritance for the gene for albumin proteins, and with a mating call that was intermediate between the two. Morphology was examined for over five hundred specimens collected during our period of fieldwork, a study that provided the first indication of widespread hybridization and introgression in the sample. This was supported by subsequent studies that compared mating call, albumin protein mobility, DNA volume and osteology. Specimens were also found to be readily inter-fertile, enabling F1 hybrids and F2-backcross individuals to be raised in the laboratory for comparison with wild-caught specimens. The field-sample was found to comprise a large number of intermediates. Evidence of genetic introgression was found even in specimens deemed to have been pure *X. l. laevis* or *X. muelleri* on account of external morphology. That even apparently pure taxa possessed introgressed genes provided a possible explanation for results obtained in chapter 6, which compared *X. muelleri* between this and nearby localities. It is likely that introgressed genes extend to contaminate the gene pool even in allopatric sites. This need not compromise the genetic fitness of either population however (Fischer *et al.*, 2000). Indeed, a degree of genetic contamination may even increase the range of ecological tolerance and adaptive plasticity of a taxon (Picker, Harrison and Wallace, 1996). Furthermore, the ranges of each of the two taxa in this hybrid zone are so broad that is remains unlikely that the effects of hybridization will ever infiltrate right through the distribution range of the species.

The final study examined endemic taxa originating from Ethiopia, a country that is in stark contrast to those which have formed the biogeographical focus of preceding chapters. Despite having acted as an ice-age refugium, Ethiopia is characterized by striking species poverty whilst boasting a remarkably high proportion of endemic taxa. Two main factors have contributed to this unusual biogeographic pattern. A longstanding history of aridity is explained by the sheer extent of high altitude land: south of the tropic of Cancer, nearly 80% of land over 3000m lies within its borders (Largen, 1998). Of equal significance is its isolation from other highland areas by the formidable A*cacia-Commiphora* scrub-desert region (Olson *et al.*, 1998) that extends around its southern margin preventing migration of taxa, even during interglacial periods of the Pleistocene, in all probability (Hamilton, 1982). Only two *Xenopus* taxa are known from Ethiopia, *X. clivii* and *X. largeni*. Whilst reports regarding the taxonomic position of *X. largeni* are somewhat contradictory, with some accounts allying the taxon with *X. laevis* and others demonstrating it to be taxonomically isolated with respect to the remainder of the genus, that of *X. clivii* is generally considered to be isolated

(Graf, 1996; Kobel, Barandun and Thiebaud, 1998). Whilst it was not possible to examine the osteology of *X. clivii,* x-rays were obtained of this taxon, and showed atlas and axis to be separate, confirming its taxonomic position within *Xenopus*. Interestingly however, of the sample of three borrowed from the British Museum of Natural History, one male specimen possessed keratinized spinules, only otherwise found in *Silurana* and in *X. longipes*, the only *Xenopus* taxon currently documented as being taxonomically intermediate between sub-genera. This finding indicates that the taxon currently referred to as *X. clivii* in fact comprises at least two taxa. Osteological preparations of *X. largeni* revealed that it possesses a number of osteological features which place it in a hitherto-unknown group that is intermediate between the two *Xenopus* sub-species, *Silurana* and *Xenopus*. Furthermore, that atlas and axis are fused distinguishes it unambiguously from *X. clivii*, its closest geographic neighbour. These results suggest that the split between *Xenopus* sub-genera is perhaps not so profound as had previously been indicated.

In addition to the opening osteological account, which provides an overview of osteology in the genus *Xenopus*, the osteology of each of the taxa that have formed the basis of subsequent chapters has been described for the first time. This provides the potential for a reassessment of the taxonomic position of a number of fossil taxa whose positions are considered ambiguous (see Baez, 1996), thereby enabling a reconsideration of the former distribution range of the genus.

Results from a number of chapters are consistent with those from previously published accounts of taxonomic relations in the genus, qualifying the use of comparative osteology as a method to these ends. In many respects, the method is preferable to others owing to the straightforward procedure involved in preparation of skeletons, the speed at which data can be gathered, the repeatable nature of data acquisition and the availability of a large number of specimens which exist in university and museums collections worldwide. Furthermore, the method circumvents having to sacrifice live material. Moreover, osteology is under polygenic control, and perhaps provides a more sensitive taxonomic scale by which taxa may be compared. The method may be applied in favour of those which employ matrilinearly inherited material for inter-taxon comparison, the limitations of which are obvious, or those which compare genetic expression at a selected number of loci. Data are also available for ultimate incorporation into wider studies whose aim is to assess the position of fossil taxa within the context of extant species.

BIBLIOGRAPHY

Bàez, A.M. 1996. "The fossil record of the Pipidae." Pp. 329-47 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press

Burki, E. and Fischberg, M. (1985). Evolution of globin expression in the genus *Xenopus* (Anura: Pipidae). *Molecular Biology and Evolution* **2**:270-277.

Cannatella, D.C. and Trueb, L. (1988). Evolution of pipoid frogs: Morphology and phylogenetic relationships of *Pseudhymenochirus*. *Journal of Herpetology* **22** (**4**):439-456.

Carr, S.M., Brothers, A.J. and Wilson, A.C. (1987). Evolutionary inferences from restriction maps of mitochondrial DNA from nine taxa of *Xenopus* frogs. *Evolution* **41**: 176-188.

De Klerk, H.M., Crowe, T.M., Fjeldsa, J. and Burgess, N.D. (2002). Patterns of species richness and narrow endemism of terrestrial bird species in the Afrotropical region. *Journal of Zoology, London* **256**:327-342.

Elepfandt, A. 1996. "Underwater acoustics and hearing in the clawed frog *Xenopus*". Pp. 177-191 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Fischer, W.J., Koch, W.A. and Elepfandt, A. (2000). Sympatry and hybridization between the clawed frogs *Xenopus laevis laevis* and *Xenopus muelleri* (Pipidae). *Journal of Zoology, London* **252**:99-107.

Graf, J.-D. 1996. "Molecular approaches to the phylogeny of *Xenopus*". Pp. 379-388 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Gurdon, J.B. 1996. "Introductory comments: *Xenopus* as a laboratory animal". Pp. 3-6 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford. Clarendon Press.

Hamilton, A.C. ed. 1982. Environmental History of Africa: A Study of the Quaternary. London: Academic Press.

Kobel, H.R., 1996. "Allopolyploid speciation." Pp391-400 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Kobel, H.R., Barandun, B. and Thiébaud, ChH. (1998). Mitochondrial rDNA phylogeny in *Xenopus*. *Herpetological Journal* **8** (**1**):13-18.

Kobel, H.R., Loumont, C and Tinsley, R.C. 1996. "The extant species." Pp. 9-33 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Largen, M.J. (1998). A preliminary review of the amphibians of Ethiopia. *The Herpetological Journal* **8**(**1**):7-12.

Loumont, C. and Kobel, H.R. (1991). *Xenopus longipes* sp. nov., a new polyploid pipid from western Cameroon. *Revue Suisse Zoologie* **98** (**4**):731-738.

Measey, G.J. (1997). Ecology of feral *Xenopus laevis*. Ph.D dissertation, University of Bristol.

Olson, D.C., Dinerstein, E., Wikramanayake, E.D., Burgess, N.D., Powell, G.V.N., Underwood, E., D'Amico, J.A., Itoua, I.I., Strand, H.E., Morrison, J.C., Loucks, C.J., Allnutt, T.F., Lamoreux, J.F., Ricketts, T.H., Kura, Y., Lamoreux, J.F., Wettengel, W.W., Hedao, P. and Kassem, K. (2001). Terrestrial ecoregions of the world: a new map of life on earth. *Bioscience* **51(11)**: 933-938.

Picker, M.D., Harrison, J.A. and Wallace, D. (1996). "Natural hybridization between *X. l. laevis* and *X. gilli* in the south-western Cape Province, South Africa". Pp. 61-71 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Poynton, J.C. and Boycott, R (1996). Species turnover between Afromontane and east African lowland faunas. Patterns shown by amphibians. *Journal of Biogeography* **23**:669-680.

Reumer, W.F. (1985). Some aspects of the cranial osteology and phylogeny of *Xenopus* (Anura, Pipidae). *Revue Suisse Zoologie* **92** (4):969-980.

Schmid, M. and Steinlein, C. (1991).Chromosome banding in Amphibia XVI. High resolution replication banding patterns in *Xenopus laevis*. *Chromosoma* **101(2)**:123-132.

Smirnov, S.V. (1994). Postmaturation skull development in *Xenopus laevis* (Anura, Pipidae): Late-appearing bones and their bearing on the pipid ancestral morphology. *Russian Journal of Herpetology* **1, 1**:21-29.

Tinsley, R. C. (1973). Studies on the ecology and systematics of a new species of clawed toad, the genus *Xenopus*, from western Uganda. *Journal of Zoology, London* **169**:1-27.

Tinsley, R. C. (1981). Interactions between *Xenopus* species (Anura: Pipidae). *Monitore Zoologico Italiano* **9**:133-150.

Tinsley, R.C. 1996. "Parasites of *Xenopus*." Pp. 233-251 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Tinsley, R.C., Loumont, C. and Kobel, H.R. 1996. "Geographical distribution and ecology." Pp. 35-59 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.

Tinsley, R.C., Kobel, H.R. and Fischberg, M. (1979). The biology and systematics of a new species of *Xenopus* (Anura:Pipidae) from the highlands of Central Africa. *Journal of Zoology, London* **188**:69-102.

Tinsley, R.C. and Kobel, H.R., eds. 1996. The Biology of *Xenopus* 1st ed. Oxford: Clarendon Press.

Tinsley, R.C. and Jackson, J.A. (1998). Speciation of *Protopolystoma* Bychowsky, 1957 (Monogenea: Polystomatidae) in hosts of the genus *Xenopus* (Anura: Pipidae). *Systematic Parasitology* **40**:93-141.

Tymowska, J. (1977). A comparative study of the karyotypes of eight *Xenopus* species and subspecies possessing 36-chromosome complement. *Cytogenetics and Cell Genetics* **18**:165-181.

Wasser, S.K. and Lovett, J.C. 1993. "The Geological Evolution of East Africa." Pp. 30-47 in Biogeography and Ecology of the Rainforests of Eastern Africa. : Cambridge University Press.

Yager, D.D. 1996. "Sound production and acoustic communication in *Xenopus borealis*." Pp. 121-141 in The biology of *Xenopus*, edited by Tinsley, R.C. and Kobel, H.R. Oxford: Clarendon Press.